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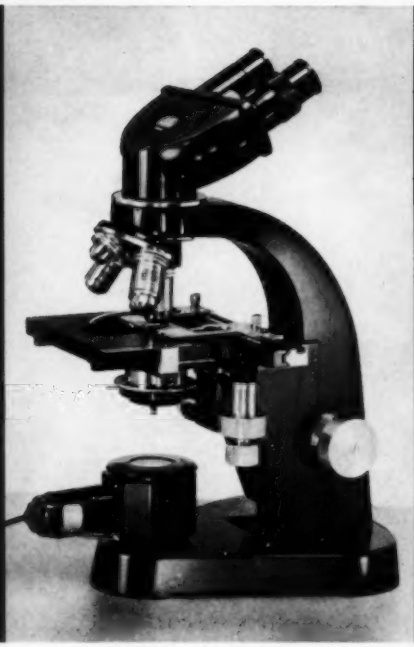

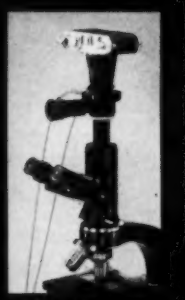

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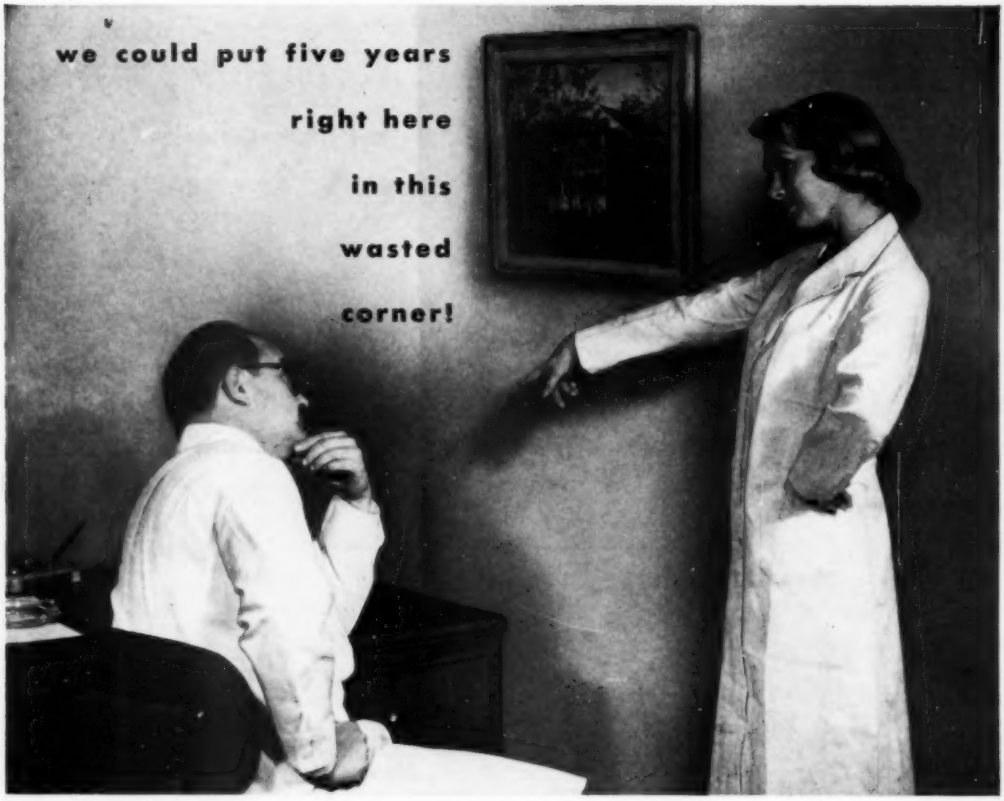

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Decompression Sickness

A Review of the Literature and Previously Unreported Histologic Observations

ROBERT J. SILLERY, M.D., Detroit

The first man who attempted to explore the mysteries of the subterranean world quickly became aware of two great problems. Namely, the need for a suitable air supply and the increased pressure upon his body. The amount of air with which he filled his lungs was sufficient only for a very limited stay beneath the surface of the water. The next step, presumably, was the use of a hollow reed to gain the necessary air supply. He placed one end of the reed in his mouth, while the other end remained above the surface of the water. This, however, was not a satisfactory solution, since his movements were extremely restricted.

Tradition has it that Aristotle conceived the idea of the diving helmet and that the contemporary ruler, Alexander the Great, descended to the bottom of the sea in a diving bell. The first authentic accounts, however, of the use of a diving bell date from A. D. 1525 when Sturmius invented a bell in which the only ventilation afforded was by the breaking of bottles of air carried in by the workers. In 1605, a diving bell was used to view parts of the sunken Spanish Armada. Since the air supply could not be replenished, many tragedies occurred as a result of these experiments. Curiously, many of the survivors experienced a syndrome of a generalized muscle, joint, and abdominal pain, which caused them to double up. Sometime later, this characteristic posture was given the vernacular name of the "bends." At first it was believed that the syndrome was related to a lack of oxygen.[1] The puzzling fact, however, was the sudden death of some of these victims within minutes to hours after reaching the surface, where sufficient oxygen was available.

In 1839, Triger, a French engineer, devised a method for delivering air under pressure to the caisson which he invented. The problem of an adequate and continuous air supply was thereby solved. It was then soon recognized that the syndrome of the bends was not related to oxygen insufficiency but rather to alteration in the atmospheric pressure. His observations of the occurrence of pain in the arms and legs of the workers constitutes the earliest mention of this new disease.

During the decade of 1870 to 1880, caissons were used in the construction of the piers for the Brooklyn Bridge and the Hudson Tubes in New York. At the bridge site, pressures of 1.5 atmospheres were used and the mortality rate among the workers was 3%. In the Hudson Tube,

Submitted for publication March 25, 1958.

Assistant Professor of Pathology, Wayne State University College of Medicine; Instructor, Wayne State University School of Mortuary Science, and Deputy Medical Examiner, County of Wayne. From the Office of the Medical Examiner for the County of Wayne, 400 E. Lafayette St.

construction pressures up to two atmospheres were employed and the mortality reached 25.0%. In 1875, the medical lock was invented. When this device was installed at the Tube site, the mortality due to decompression sickness dropped to 1%.

With the development and the use of the medical lock, the problem of decompression sickness was thought to be practically solved.[2] Unfortunately, this is not the case, for when workers are subjected to increased atmospheric pressures, several cases may be seen in a working day. In most instances, these men recover and return to work. There are, however, occasionally fatal cases. A review of the literature discloses some reports of deaths followed by necropsies. Such reports, however, are absent from the recent literature.

It is the intent of this paper to present a fatal case of decompression sickness with a review of the literature describing autopsy findings in similar cases. It is also our intent to present histologic observations, which have not been previously reported.

Review of Literature

Reports of autopsied cases are limited. The records are also sketchy, and in many instances the necropsy was conducted many hours to days after death. Refrigeration was not employed, and putrefactive gas formations may have obscured the findings.

Heiberg[3] autopsied a worker who died within a few hours of decompression. The skin of the chest, back, and abdominal wall was covered with multiple livid reddened patches and was emphysematous to palpation. The inferior vena cava contained a clot filled with trapped air bubbles. Air bubbles were also seen in the right ventricle of the heart and in the liver, spleen, and intestines. The lungs were congested, and there was a thrombus in a vessel in the lower lumbar spinal cord. Altschul[4] autopsied dogs which he rapidly decompressed after keeping them at 43.5 m. below sea level and noted air bubbles in almost all of the intestinal organs and the central nervous system. Heller[5] reported an autopsy on a young man who died two hours after "locking out." Bubbles were found in the meningeal vessels, and the lungs were edematous. This necropsy, however, was performed 32 hours after death. Schäffer[6] reported 135 fatal cases, of which only 18 were autopsied. Nine of these necropsies showed air bubbles in the abdominal cavity, as well as in the blood vessels. McKinlay[7] and Rudge[8] each reported the death of a diver showing air bubbles in the venous system. Oudard[9] necropsied a young caisson worker 64 hours after death and found bubbles in the coronary arteries, the aorta, and the venous system. Erdman[10] reported the findings of seven autopsies which consisted of bubbles noted in the blood of the victims but little else.

Case History

A 35-year-old white man had been working under increased pressure in a caisson for some six hours. This was two to three hours over the usual period. He passed through the medical air lock along with several other men, who experienced no subsequent symptoms. He returned home, but before he could enter the house he experienced generalized pain and vertigo. He was returned to the medical lock, where he was recompressed and slowly decompressed over a period of one and one-half hours. This, however, did not relieve his distress, and he was admitted to a hospital. On arrival at the hospital, the patient was a well-developed, slightly obese, white man in coma. Respiration was labored; no blood pressure could be obtained, and he died within 10 minutes of his entry.

Autopsy

The body was refrigerated. The autopsy was performed seven hours after death. Externally, a diffuse red discoloration of the skin of the upper thorax, shoulders, neck, face, and head was quite striking. The chest and abdomen were crepitant. On section, most of the blood vessels contained minute air bubbles, causing the blood to be frothy.

The body cavities were smooth and glistening throughout, and the visceral organs maintained their usual positions and relations. The heart was moderately hypertrophied, weighing 400 gm. It was also crepitant to palpation. The pericardium was filled with water, and when the cardiac cham-

bers were incised, large bubbles escaped from the right atrium and the inferior vena cava. The leaflets of the cardiac valves were thin and intact. The myocardium was firm and reddish-brown in color. The coronary arteries were widely patent, and their intimal surfaces were smooth.

The lungs were somewhat heavier than usual, each weighing 525 gm. They were subcrepitant throughout. On section, a small amount of frothy fluid ran from the surface and a moderate amount of blood escaped from the vessels. The bronchi and blood vessels were devoid of gross changes.

The liver weighed 2050 gm. It was very yellow in color and crepitant throughout. On section, it had a definite yellow color and a glistening oily surface.

The gallbladder and biliary passages displayed no macroscopic changes.

The spleen weighed 190 gm. and showed some congestion. The pancreas and adrenals displayed no gross changes.

The left kidney weighed 150 gm.; the right, 130 gm. The gross architecture of each kidney was well preserved. However, there was some congestion. The remainder of the genitourinary tract displayed no gross changes.

The gastrointestinal tract was remarkable only for the fact that the omentum and the mesentery were crepitant.

Examination of the brain was carried out. The brain weighed 1350 gm. No macroscopic changes were noted in the brain.

Microscopic Examination

The histologic observations proved to be of great interest. In the lungs, bubbles were seen within large pulmonary arteries (Fig. 1). The alveoli contained some serous fluid, red blood cells, lymphocytes, and pigment-laden macrophages. In the liver most of the cells contained lipoid vacuoles, and there were bubbles within the large blood vessels. There were numerous lipoid droplets within the parenchyma of the pancreas. The brain showed congestion of blood vessels throughout and small perivascular hemorrhages in the cerebral cortex. Within an occasional blood vessel there were small bubbles.

The intracerebral blood vessels about the internal capsule and basal ganglia showed deposition of calcium in their walls. The spinal cord showed only some congestion of blood vessels. The heart exhibited slight

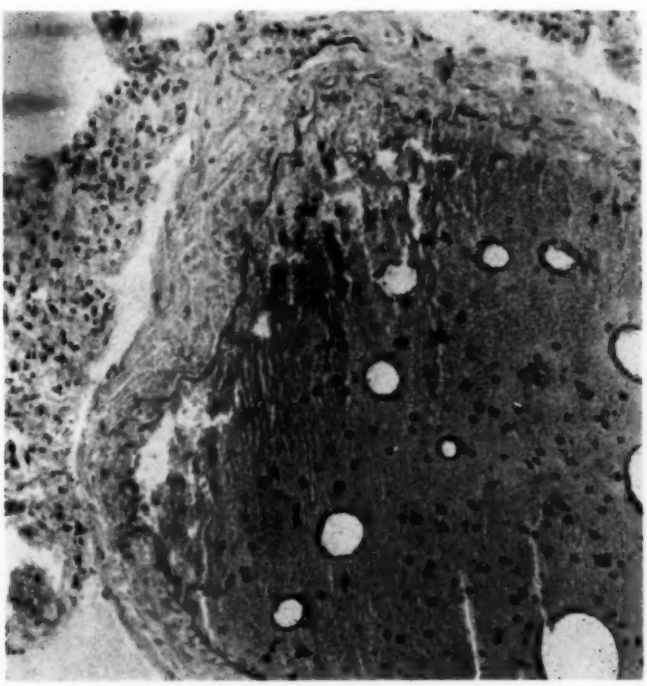

Fig. 1.—Cross sections of pulmonary vein, showing gas bubbles in the blood. Hematoxylin and eosin; reduced 6% from mag. × 350.

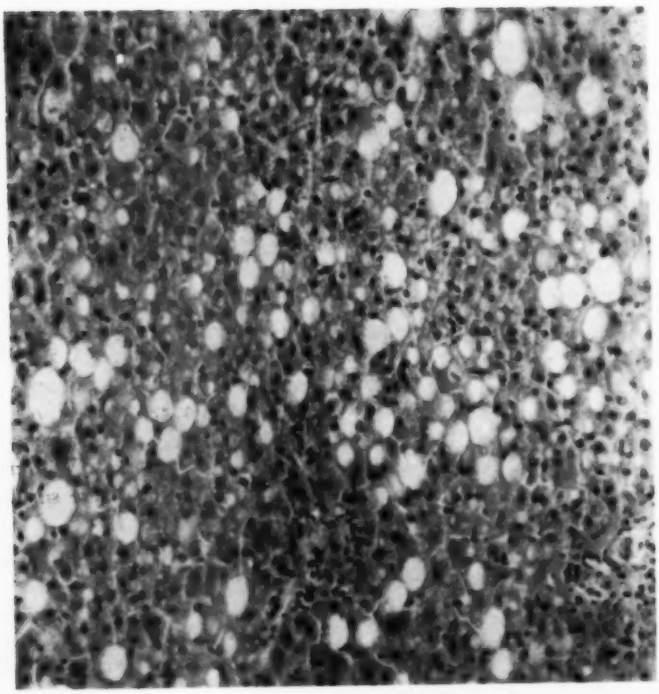

Fig. 2.—Section of liver, showing fatty metamorphosis. Hematoxylin and eosin; reduced 6% from mag. ×100.

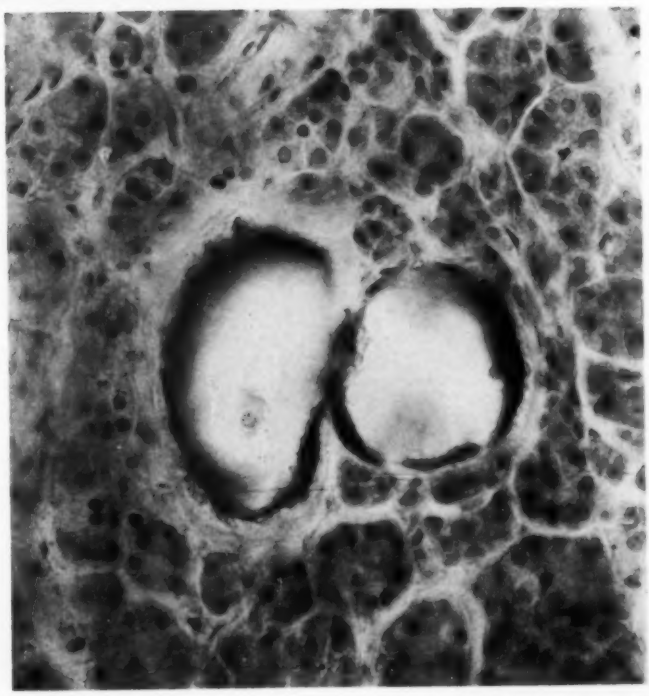

Fig. 3.—Section of liver, showing gas bubbles within lipid. Sudan IV; reduced 6% from mag. ×450.

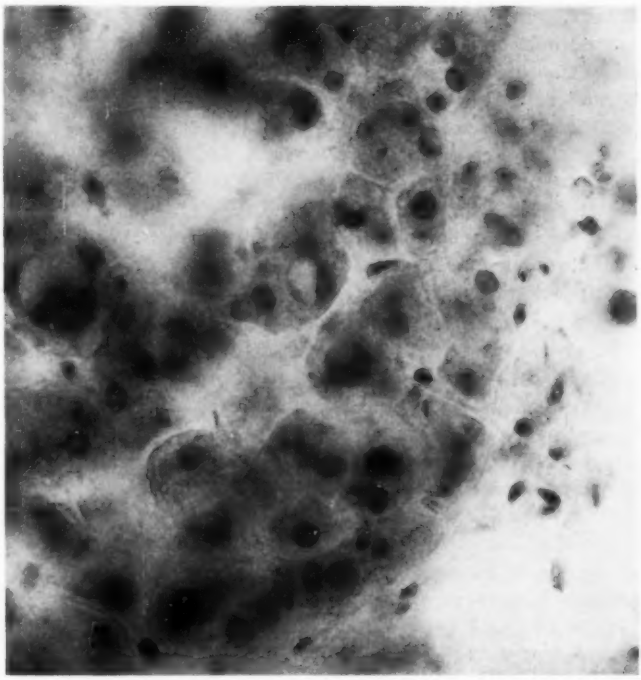

Fig. 4.—Section of pancreas, showing gas bubbles within lipid droplets. Lipid stain dark. Sudan IV; reduced 6% from mag. × 450.

hypertrophy and congestion of coronary veins. There was congestion of blood vessels throughout the kidneys. The architecture of the glomeruli and tubules was well preserved. Sections of skin, subcutaneous tissue, and striated muscle were taken. These presented congestion of blood vessels, with an occasional bubble in the blood. As a routine, all tissues were stained with hematoxylin and eosin. However, sections of pancreas and liver were stained with Sudan IV for fat. Large amounts of fat were demonstrated in the liver and pancreas. In the liver (Fig. 3) and pancreas (Fig. 4) there were seen bubbles within the lipoid masses.

Comment

It is evident that autopsy reports of deaths due to acute decompression sickness are quite incomplete and histologic observations, absent. The histologic demonstration of bubbles within fat is extremely interesting.*

One may inquire as to the composition of these bubbles. The glib statement is often made that they are nitrogen. In 1873, Paul Bert [11] a French physiologist, subjected innumerable animals to increased atmospheric pressures. He then suddenly decompressed them in order to reproduce the syndrome of decompression sickness, which is produced as a result of decompression. He autopsied these animals and analyzed the gas bubbles:

Pressure	Oxygen	Carbon Dioxide	Nitrogen
10A	Trace	20.8	79.2
9.5	2.0	15.2	82.8

* It is true that a similar appearance may be simulated by nonstainable lipoid. In our material, numerous attempts to stain these "bubbles" were unsuccessful. This, combined with the gross observation of crepitation in the liver and other solid organs and the fact that gas bubbles can be expressed from these organs under water, clearly indicated that these are gas bubbles, not nonstainable lipoid.

Bert demonstrated that these bubbles contain only a trace of oxygen and a large proportion of nitrogen but also a significant amount of carbon dioxide. The histologic presence of these bubbles within lipid graphically demonstrates the well-known fact of the solubility of nitrogen in this substance. We must add to this the solubility of carbon dioxide. These observations have not been made previously on human material.

Summary

Histologic observations of the effects of increased atmospheric pressure on human tissues are made which were hitherto unreported. The literature concerning reports of autopsies on deaths due to acute decompression sickness is reviewed. It is concluded that the exact chemical composition of the gas bubbles encountered in this disease has been oversimplified.

Office of the Medical Examiner for the County of Wayne, 400 E. Lafayette St. (26).

REFERENCES

1. Biggar, H. F.: "The Bends": Caisson Disease, or Diver's Paralysis, M. Century 8:203-209, 1900.

2. Brooks, H.: Caisson Disease: The Pathological Anatomy and Pathogenesis, with an Experimental Study, Long Island M. J. 1:149-158 and 196-208, 1907.

3. Heiberg, D.: Autopsie d'un malade mort en sortant de l'air comprimé, Gaz. méd. Paris, (s. 5) 7:540, 1878.

4. Altschul, A.: Beitrag zur Kasuistik der Taucherkrankheiten, Wien. med. Wchnschr. 45: 1977-1982 and 2020-2026, 1895.

5. Heller, R.; Mager, W., and von Schrotter, H.: Zur Kenntniss der Todesursache von Pressluftarbeitern aus den Untersuchungen über "Luftdruckerkrankungen," Deutsche med. Wchnschr. 23: 375-379, 1897.

6. Schäffer, E.: Sektionsbefunde bei Pressluft-(Caisson-) Arbeitern, Ztschr. Med.-Beamte 11: 389-397, 1898.

7. McKinlay, A.: Case of Death from Syncope Caused by Caisson Disease, in Gt. Britain: Statistical Report of the Health of the Navy for the Year 1900, London, Eyre and Spottiswoode, 1901, Vol. 9, Appendix, pp. 102-104.

8. Rudge, F. H.: A Case of "Caisson Disease," Lancet 2:1675-1676, 1907.

9. Oudard: Accidents de décompression, rélation d'autopsie, Arch. méd. pharm. nav. 96:63-72, 1911.

10. Erdman, S.: Aeropathy or Compressed Air Illness Among Tunnel Workers, J. A. M. A. 49: 1665-1670, 1907.

11. Bert, P.: La Pression barométrique, Paris G. Masson, 1878.

Acceleration of Intimal Atherogenesis Through Prior Medial Injury

P. CONSTANTINIDES, M.D., Ph.D.; N. GUTMANN-AUERSPERG, M.D., and D. HOSPES, Vancouver, B.C., Canada

Introduction

In the course of a previous study in this laboratory,[1] it was observed that the aorta of some rabbits that developed accidental renal damage while fed cholesterol exhibited patchy media necrosis and an unusually severe degree of diffuse intimal atherosclerosis, as judged by a battery of quantitative criteria.

Since no atherosclerosis occurred in the aorta of a few rabbits that developed renogenic medial injury while given a normal diet, it was postulated that focal medial damage greatly augments the atherogenic response of the intima to lipemia.

The present study was designed to test the above hypothesis (which was based on accidental findings) by specifically planned experimentation. Aortae with prior drug-induced medial injury were exposed to lipemia and compared with normal aortae exposed to lipemia of the same degree and duration as well as with medially injured aortae not exposed to any lipemia. Aortic media necrosis was produced with combined epinephrine and thyroxin overdosage, a treatment which, although highly toxic, proved far more consistent in causing medial injury in the rabbit than a number of other agents (epinephrine alone, Na_2HPO_4, sulfathiazole, parathyroid hormone) tested in preliminary experiments.

Submitted for publication March 3, 1958.

From the Anatomy Department, University of British Columbia Faculty of Medicine.

The results of this study were presented at the 2d annual meeting, in Seattle, of the Northwestern Section, Society for Experimental Biology and Medicine, Nov. 9, 1957, and at the 12th annual meeting, in Banff, Alta., Canada, of the Western Section, Medical Division, National Research Council of Canada, Jan. 30, 1958.

Materials and Methods

A lot of 28 New Zealand White rabbits were given a normal diet and daily intravenous injections of epinephrine (Parke, Davis & Company, 1:1000 solution) at 0.05 mg. per kilogram for 10 days. During the last five days of the intravenous treatment period, the animals were also given daily subcutaneous injections of an aqueous suspension of thyroxin (B. D. H., crystalline), at 1 mg. per kilogram. Ten rabbits died in the course of the above injection period and were discarded.

The 18 survivors were then submitted to a four-day rest interval, during which another 9 rabbits died. These latter nine animals constituted Group I of the present experiment ("medial injury only"), and their aortae, hearts, and kidneys were sampled in a standard manner.

After the end of the rest interval, the remaining 9 survivors (from the original lot of 28 epinephrine- and thyroxin-treated animals) were given a 1% cholesterol and 5% cottonseed oil diet for three weeks and then killed, constituting Group II ("medial injury followed by lipemia").

Simultaneously, nine normal untreated rabbits were given a 1% cholesterol and 5% cottonseed oil diet for three weeks and then killed, constituting Group III ("lipemia only").

After autopsy, the aorta (from the valves to the iliac bifurcation), the whole heart, and the whole left kidney from every animal in every group, as well as a sample from the right lobe of the liver of every animal in Group I, were removed for further studies.

Each aorta was stripped of its adventitia and weighed, and the total area of its grossly visible media-necrosis patches and/or atheromata—if any—was determined planimetrically from drawings. A small sample, about 3×5 mm., was taken from the center of the thickest atheroma or, if no atherosclerosis was visible, from the largest media-necrosis patch of every aorta. All samples were fixed in 10% aqueous formalin and processed to

TABLE 1.—*Blood Lipids and Urea* *

	Plasma Turbidity, Klett Units			Plasma Cholesterol, Mg. %	Plasma Total Lipids, Mg. %	Blood Urea, Mg. %	
	Day 0	Day 11	Day 19	Day 21	Day 16	Day 0	Day 19
Group I (vascular injury only)	--	--	--	--	--	18±1.5	--
Group II (vascular injury plus lipemia)	5.2±0.3	15.0±2.3	22.8±3.0	777±130	1,009±134	17±2.8	10±0.8
Group III (lipemia only)	4.4±0.2	13.8±1.7	27.3±5.3	861±155	1,078±136	--	10±1.1

* Mean ± *E*. The days are numbered with reference to the commencement of cholesterol feeding.

15μ thick Sudan red- and hematoxylin-stained frozen sections in which maximal plaque thickness (from internal elastic membrane to crest of the atheroma) was determined micrometrically, in microns. Additional toluidine blue-, colloidal iron-, periodic acid-Schiff- and von Kossa-stained paraffin sections were prepared from alcoholic formalin-fixed specimens where indicated. After removal of the histology sample, the total cholesterol content of every aorta was determined as previously described.[2]

The other organs were fixed in 10% formalin. Coronary atherosclerosis was assessed in accordance with a previously reported procedure,[2] and routine histological studies were made in hematoxylin-and-eosin-stained paraffin sections of the kidney and liver.

At various intervals during the experiment, citrated blood samples were obtained from the ear veins of all animals for the following determinations: (*a*) plasma turbidity and cholesterol, as previously described,[2] (*b*) plasma total lipids, by the method of Swahn,[3] (*c*) blood urea, by the method of Karr.[4]

Results

1. *Blood* (Table 1).—The two cholesterol-fed groups (Groups II and III) developed a progressively increasing lipemia at the same rate and achieved the same plasma turbidity, total cholesterol, and total lipid level at the end of the three-week cholesterol-alimentation period. Blood urea was significantly elevated at the end of the epinephrine and thyroxin treatment period (in Groups I and II), but it returned to normal levels after discontinuation of the injections (in Group II).

2. *Aorta* (Table 2 and Figs. 1-3).—(*a*) Media Necrosis: This could be easily distinguished from atherosclerosis. It consisted of multiple white or gray patches which were usually hard and could often be palpated from the outer surface of the aorta. In a good number of cases, these patches had an elevated rim and a thinned-out, depressed, sometimes almost aneurysmatic center; many such confluent lesions gave the aorta a "snake-skin" or "tree-bark" appearance, and they were most frequently encountered in the lower thoracic and abdominal aorta. Microscopically, these medial lesions consisted of focal deposits of an amorphous basophilic material (presumably an acid mucopolysaccharide on the basis of its metachromasia to toluidine blue and its colloidal-iron positivity), distortion and disintegration of elastic laminae, variable calcification of the basophilic material, and mononuclear infiltration (Fig. 1); they were always located in the inner (subintimal) third or half of the media.

Both groups given injections of epinephrine and thyroxin (Groups I and II) developed media necrosis, with 100% incidence and of approximately the same se-

TABLE 2—*Aorta* *

	Incidence of Atherosclerosis	Maximal Atheroma Thickness, μ	Total Cholesterol Content, Mg/Aorta	Total Area of Medial Necrosis, % of Total Aortic Area	Wet Weight, Mg.
Group I (vascular injury only)	0/9	--	1.01±0.2	22.5±5.2	702±54
Group II (vascular injury plus lipemia)	8/9	60±17	4.0 ±1.2	30.2±7.9	1126±66
Group III (lipemia only)	0/9	--	1.17±0.2	1.5±0.6	805±44

* Mean ± *E*.

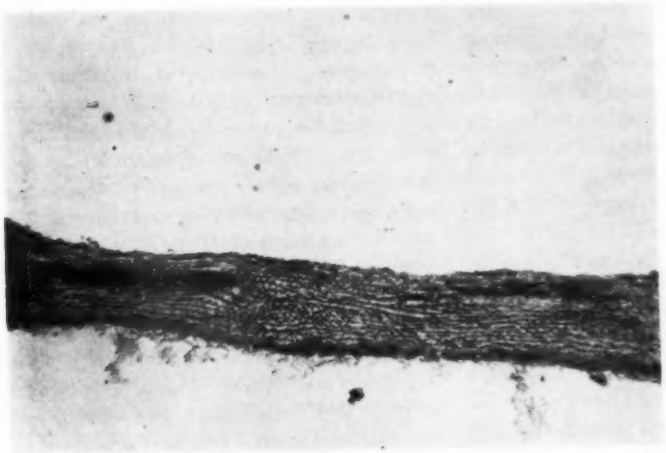

Fig. 1.—Typical abdominal aorta from Group I (medial injury only). Note the two dark streaks in the media that represent foci of injury and consist of basophilic deposits in areas of beginning elastic disintegration, the lesion on the left being the severer one. There is no trace of intimal atherosclerosis above or adjacent to these medial lesions. Frozen section, Sudan red and hematoxylin; × 49.

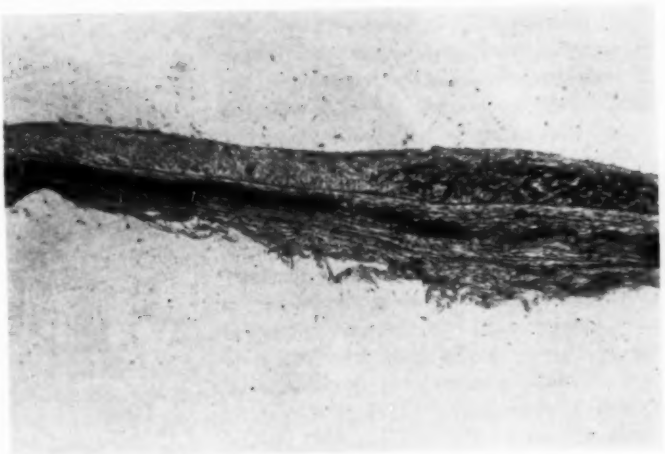

Fig. 2.—Typical abdominal aorta from Group II (medial injury followed by lipemia). Note the development of considerable intimal atheromatosis over the entire strip of medial injury. The left half of the atheroma that overlies the more severely damaged part of the media (black band) is lipid-free, whereas the right half that overlies the least affected portion of the media contains appreciable amounts of sudanophilic lipid (reticular-appearing dark material). Frozen section, Sudan red and hematoxylin; × 49.

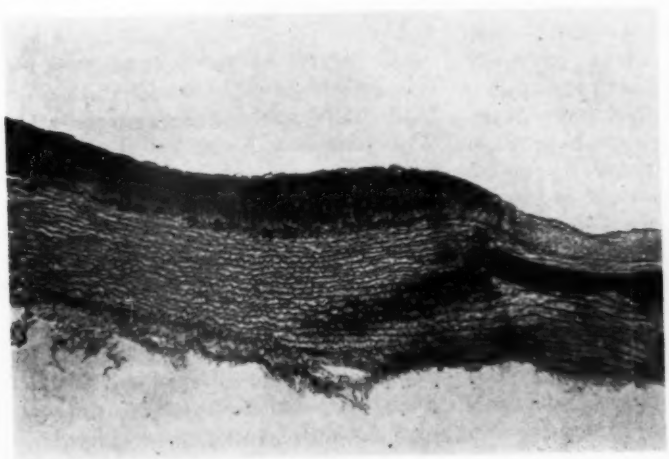

Fig. 3.—Part of an injury-associated atheroma in a thoracic aorta from Group II. The atheroma extends not only over the medial injury strip (partially visible as a black band in the right end of the field) but also over a considerable stretch of apparently normal adjacent media. Note the migration of some lipid from the plaque into the media immediately adjacent to the injury strip as well as the absence of lipid from the portion of the atheroma that directly overlies the visible medial damage. Frozen section, Sudan red and hematoxylin; × 49.

vere degree, whereas the group not given injections (Group III) exhibited no more than the negligible amount of "spontaneous" media necrosis found in the aortic arch of most rabbit populations. Thus the regular incidence of media necrosis after the combined drug treatment was in good agreement with the findings of Oester et al.[5]

(*b*) Atherosclerosis: Of the two groups that were exposed to three weeks' cholesterol feeding, only the one whose aortae had been previously injured through the drug treatment (Group II) developed atherosclerosis, with 89% incidence. No atherosclerosis developed in the cholesterol-fed animals that had no prior vascular injury (Group III) or in those that were submitted to injury only (Group I). These relationships were clearly reflected in the aortic cholesterol and aortic wet-weight values, which were both significantly higher in Group II than in Group III (by approximately 240% and 40%, respectively), while there was no significant difference between Groups III and I with regard to these two criteria. Since many of the intimal lesions in Group II were thin and most were overlapping with the medial lesions, the estimations of the total atheroma area of each aorta were only approximate and were not tabulated.

Microscopically, the atheromata in Group II were mostly overlying the patches of necrotic media, but some also extended over the apparently normal neighboring media (Figs. 2 and 3); indeed, in one instance, atheromatous plaques were encountered without light microscopic evidence of structural changes in the underlying media. Some of the atheromata were remarkable for their thickness, which was of a magnitude normally not achieved before the eighth week of 1% cholesterol feeding. There was a tendency for the central area of the intimal plaques (the one directly overlying the medial injury focus) to be lipid-poor or lipid-free; in some instances, this was seemingly due to migration of sudanophilic material from the atheroma into the underlying damaged media, but in others no such migration was visible. Also, in a number of cases, the central part of the atheromata was thinner than their rim, giving them a somewhat umbilicated appearance.

3. *Heart.*—A few coronary atheromata were seen in the standard heart sections of four out of the nine animals of Group II (44% incidence), but none were encountered in the other two groups. No media necrosis was seen in any of the coronaries of the examined sections of the three groups.

4. *Liver and Kidney.*—Some centrilobular necrosis and fatty change was observed in the livers of Group I (the only group whose liver was examined microscopically). There was a very slight degree of tubular lipoidosis in some kidneys of the groups given injections of epinephrine and thyroxin, unaccompanied, however, by any real histological evidence of renal damage.

5. *Clinical Condition, Body Weight.*—The combined epinephrine and thyroxin treatment was very toxic, as evidenced by the high mortality among the original animals given injections and by the catabolism in Groups I and II. These two groups lost an average of 8.2% and 10.2%, respectively, of their initial body weight during the experiment, while Group III gained 18%.

Comment

The above data demonstrate unequivocally that, through some unknown mechanism, prior medial injury greatly increases the atherogenic response of the aortic intima toward a hypercholesteremia. The areas of directly overlying the foci of medial injury intima so sensitized are not only those but also those adjacent to them.

In part, therefore, the present study supports with quantitative, statistically analyzed end-points and with a critical experimental arrangement a concept that was first advanced by the Russian group of investigators[6] and subsequently reiterated by Duff,[7]

Taylor,[8] Waters,[9] and others on the basis of qualitative observations.

It is also in harmony with a number of recent qualitative findings showing that conditions associated with medial damage induced the development of intimal atherosclerosis in refractory species or increased atherogenesis in susceptible species, although the critical role of the medial involvement has not always been recognized or proven. Thus, Wissler[10] succeeded in producing coronary atherosclerosis in the rat—an animal refractory to the development of this disease in response to lipemia only—by combining dietary manipulation with renal damage (a well-established media-necrosis-inducing condition[11,12]), and Wilgram[13] accomplished somewhat similar results in the same species by combining lipemia with vitamin D overdosage (a treatment long known to cause media necrosis and calcification[6]). Similarly relevant are the histological findings of Taylor[8] and of Trueheart et al.[14] in the rabbit, who found selective development of atherosclerosis over areas of mural injury caused by local freezing and vitamin D, respectively. Also along the same line are the gross observations of Oester et al.,[15] indicating some synergism between epinephrine-thyroxin treatment and simultaneous intravenous cholesterol injections in the rabbit, as well as the histological studies of Waters,[9] showing localization of atherogenesis at sites of allylamine-induced medial damage in the coronaries of dogs given infusions of lipoprotein. Finally, there are the statistically supported reports that uranium-induced[16] renal damage accelerated and infection-associated kidney damage[1] greatly increased aortic atherogenesis in lipemic rabbits, although media necrosis was histologically looked for and verified only in the latter study.

The present data are of theoretical interest for two reasons: (1) because they reemphasize a possible mechanism whereby atherosclerosis can develop in the absence of conspicuous or prolonged blood lipid elevation, thus weakening one of the main arguments against the participation of filtration processes in the pathogenesis of atherosclerosis that "the fat in the blood does not always correlate with the fat in the arteries"; (2) because they may help explain the pathogenesis of some lipid-poor human plaques that have been described as underlaid by foci of medial elastic destruction[17] and acid mucopolysaccharide deposition,[18,19] as well as the marked so-called "secondary atherosclerosis" known to accumulate selectively over aortic areas with syphilitic media destruction.

Since an extraordinary number of different agents and conditions (including such diverse principles as corticotropin [ACTH],[20] unsaturated fatty acid deficiency,[21] vasoconstrictors,[22] and infections[6]) have been shown to produce media necrosis in mammalian arteries, medial injury could represent a "final common pathway" through which a multitude of stresses might sensitize the intima to blood lipids and thus promote atherogenesis even in the presence of only slight and transitory hypercholesteremia. Viewed in this light, factors affecting mural integrity and thereby intimal sensitivity would appear at least as important as abnormal blood lipid patterns. Should medial injury (with its predominantly lower aortic location) eventually prove to play a role in the pathogenesis of human atherosclerosis, it might also help to explain the greater severity of this disease in the lower (abdominal) as compared to the upper part of the human aorta—a localization pattern that has long been difficult to account for on the basis of hemodynamic relationships alone and that cannot be obtained experimentally by lipemia without medial damage.

Perhaps the greatest obstacle to the above view is, however, the absence of conspicuous medial changes underneath all human atheromata. This difficulty is somewhat diminished by the present demonstration of increased intimal atherogenesis not only over the visible medial injury patch but also over wide adjacent areas of apparently normal

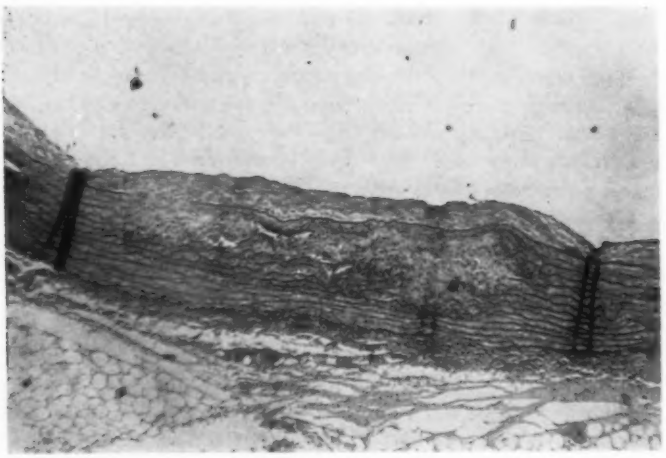

Fig. 4.—Central area of an injury-associated atheroma in the abdominal aorta of a lipemic rabbit with renal damage, from another experiment. Throughout the entire area of medial damage the elastic tissue has been destroyed and replaced by granulomatous tissue, which is still separated from the overlying atheroma by the surviving internal (first) elastic membrane. However, the latter begins to break down near the left end of the field, and as a result the intimal and medial lesions have just started to fuse. Paraffin section, hematoxylin and elastic; ×49.

media; it would be even further reduced if it could be shown that medial injuries are much more rapidly repaired than the overlying atheromata or else gradually incorporated in the latter. Indeed, there is evidence from other experiments in this laboratory that in later stages medial lesions can break down completely, fusing with the overlying intimal plaques, and that some originally "subatheromatous" calcium deposits may become "intra-atheromatous" through this process (Figs. 4 and 5). Thus, what started as an uncalcified atheroma resting on a partly necrotic calcified media may end as a calcified atheroma resting on an apparently normal (though thinner)

Fig. 5.—Injury-associated atheroma in the aortic arch of a lipemic rabbit with infection and catabolism, from another experiment. The large area of medial elastic destruction is outlined by the remaining intact media below, the frayed ends of elastic lamellae at its left and right extremity, and the surviving "ghost" of the internal (first) elastic membrane above; it contains several calcinous deposits (black masses), in addition to granulomatous tissue. It can be clearly seen that although all calcium deposits originated within the area of medial injury, they are being "incorporated" into the overlying atheroma. The two irregular cracks are microtome artifacts, caused by the brittleness of the tissue. Paraffin section, Krajian's elastic stain combined with von Kossa's calcium stain; ×49.

media. Nonetheless, this whole problem will be settled definitively only by serial studies of the natural history of experimental "injury-associated atheromata" as well as by careful surveys of the media underneath human plaques (including histochemical and electron microscopic methods) for possible changes too subtle to be noticeable on conventional examination.

Finally, attention must be drawn to certain peculiar modalities of these injury-associated plaques—notably the frequent absence of lipid from their central portion and the often pronounced migration of the sudanophilic material from the plaque into the underlying damaged media. While both phenomena seem to be sometimes interrelated and possibly the result of a local increment of mural permeability, further studies are required for their clarification. At any rate, the present findings lend further support to the revision [1] of our previous interpretation of certain atheromata with similar modalities overlying patches of calcified media, which were once encountered sporadically in some lipemic animals that had been treated with toxically high amounts of sulfated polysaccharides [2]; such lesions now appear to have been new injury-associated atheromata rather than regressing preestablished plaques, as once tentatively considered. Also of interest is the fact that some of these predominantly fibrous, lipid-poor, injury-associated atheromata (Fig. 2) are more similar to the human atherosclerotic lesions than the tightly lipid-packed plaques produced by prolonged lipemia in the absence of vascular injury.

Summary

Focal medial injuries were produced in the aortae of rabbits by treating them with toxic amounts of epinephrine and thyroxin for 10 days.

When such animals with preinjured aortae were subsequently exposed to a moderate alimentary hypercholesteremia for only 20 days, they developed measurable intimal atherosclerosis, whereas animals with uninjured aortae subjected to the same degree of hypercholesteremia and for the same period of time did not yet develop the disease. Atherogenesis in the former group occurred mainly in intimal regions directly overlying the foci of medial damage but also in adjacent areas overlying media with no light-microscopic evidence of structural changes.

Certain histological features of injury-associated atheromata are discussed, and evidence is presented that medial injury foci, including calcified ones, can fuse with the overlying intimal plaques, thus losing their original identity.

This study provides fresh support, with quantitative criteria, for the long-standing concept of the participation of mural injury factors in the pathogenesis of atherosclerosis.

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REFERENCES

1. Constantinides, P., and Saunders, P.: A. M. A. Arch. Path. 65:360, 1958.

2. Constantinides, P.; Saunders, P., and Wood, A.: A. M. A. Arch. Path. 62:369, 1956.

3. Swahn, B.: Scandinav. J. Clin. & Lab. Invest., Vol. 5, Supp. 9, 1953.

4. Karr, W. G.: J. Lab. & Clin. Med. 9:329, 1924.

5. Oester, Y. T.; Davis, O. F., and Friedman, B.: Fed. Proc. 15:464, 1956.

6. Anitchkow, N., in Arteriosclerosis: A Survey of the Problem, edited by E. V. Cowdry, New York, The Macmillan Company, 1933, Chap. 10.

7. Duff, G. L.: Arch. Path. 20:81 and 259, 1935.

8. Taylor, C. B., in Symposium on Atherosclerosis, Publication 338, National Research Council, 1955, p. 74.

9. Waters, L. L., in Symposium on Atherosclerosis, Publication 338, National Research Council, 1955, p. 91.

10. Wissler, R. W.; Eilert, M. L.; Schroeder, M. A., and Cohen, L.: A. M. A. Arch. Path. 57: 333, 1954.

11. Holman, R. L.: Am. J. Path. 17:359, 1941.

12. Lehr, D., and Martin, C. R.: Proc. Soc. Exper. Biol. & Med. 93:596, 1956.

13. Wilgram, G. F.: Circulation 16:500, 1957.
14. Trueheart, R. E.; Stumpe, M., and Hass, G. M.: Fed. Proc. 16:374, 1957.
15. Oester, Y. T.; Davis, O. F., and Friedman, B.: Circulation 10:609, 1954.
16. Moses, C., and Longabaugh, G. M.: Proc. Soc. Exper. Biol. & Med. 74:92, 1950.
17. Lansing, A. I., in Symposium on Atherosclerosis, Publication 338, National Research Council, 1955, p. 50.
18. Taylor, H. E.: Am. J. Path. 29:871, 1953.
19. Maher, J. A., and Schreier, L.: Circulation 16:492, 1957.
20. Wexler, B. C., and Miller, B. F.: Circulation 16:498, 1957.
21. Hill, E. G.; Warmanen, E. L.; Hayes, H., and Holman, R. T.: Proc. Soc. Exper. Biol. & Med. 95:274, 1957.
22. Hueper, W. C.: Arch. Path. 38:162, 245, and 350, 1944.

Renal Dysplasia and Pyelonephritis in Infants and Children

Part I

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The term *renal dysplasia* as it is used in this paper means congenital underdevelopment of the renal parenchyma. *Hypoplasia*, most commonly used for this anomaly, is unsuitable, as it seems to denote the condition in which the whole of one or both kidneys is abnormally small. It has been pointed out by Marshall [1,2] and Ekström [3] that the underdevelopment may be restricted to a small part of the renal parenchyma—"localized dysplasia or local hypoplasia." Secondary shrinkage, atrophy, and the total absence of the renal parenchyma, *agenesis* or *aplasia*, will not be dealt with here.

It is generally maintained that renal dysplasia and atrophy cannot be distinguished from one another, whether clinically, roentgenologically, or histologically,[4,5] although some workers have found it possible to differentiate between these two conditions by histologic examination [1,2] or even in the roentgenograms.[3]

The true incidence of local dysplasia is unknown. The incidence of dwarfed kidney has been given as 1:350 to 1:1600.[6-8] For the occurrence of dysplasia, Ekström [3] has given 1% for adults and 2% for children, figures based on a large clinical series. There is a clear female predominance.[3,5,9]

Dysplasia, both general and local, predisposes the patient for pyelonephritis.[1,3,5,8,10,11] Several authors have pointed to a causal connection between dysplasia and hypertension,[2,9,11,12] although most of the cases of renal hypertension described have presented atrophic changes of the kidneys. From the clinical aspect dysplasia would appear to be an important factor in the occurrence of urinary tract infections, whe-

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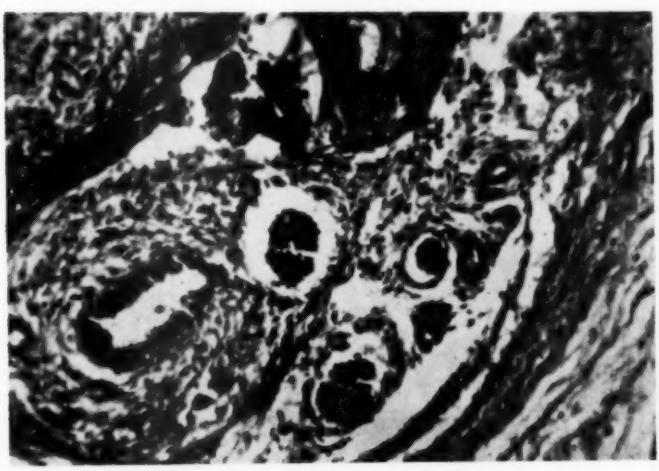


Fig. 1 (Case 13).—Grossly dysplastic kidney with primitive duct, glomeruli, and tubules. Note small ductule in upper right corner. Reduced about 10% from mag. × 160.

ther or not they are accompanied by calculi or hypertension. Such infections are very common among children, and the extent to which predisposing factors occur is of some significance,[13] particularly in the case of dysplasia.

It would appear, then, that clinical and roentgenological methods alone are insufficient for distinguishing between dysplasia and atrophy in adults who have been suffering from urinary tract infections over a long period. For demonstrating the presence of dysplasia histologic examination is essential; this is more likely to be successful in the case of children.

The purpose of the study reported in this paper was to investigate the possibilities of diagnosing dysplasia and to establish whether there is any causal relationship between this condition and infection of the urinary tract.

Material

The case series consisted of 34 children, all of whom underwent clinical and roentgenologic examinations. A pathoanatomic examination was performed on specimens obtained at operation or autopsy. Heminephrectomy was done on 23 patients and nephrectomy on 9; on 2 patients postmortem examinations were performed.

The age distribution of the material on the occasion of the examination was as follows: 1 to 7 days, 2 specimens; 1 to 6 months, 4 specimens; 7 to 12 months, 1 specimen; 1 to 2 years, 6 specimens; 3 to 5 years, 12 specimens; 6 to 8 years, 6 specimens; 9 to 11 years, 3 specimens.

The prevalence of the various clinical diagnoses was as follows: no gross anomaly, infection of urinary tract, 7; ureterocele, 16; ectopic ureter, 9; malformation of anus, 1; immaturity, 1.

The incidence of malformations of the urinary tract other than dysplasia is very high. In the case of ureteroceles and ectopic ureters there were generally duplications of the ureters and the renal pelvis on the side in question. In children these malformations are commonly found in association with chronic infections of the urinary tract; it is chiefly in such cases that surgical intervention—nephrectomy or resection—is indicated. Children presenting no such malformations very seldom undergo renal operations, as such measures are usually not necessary until adult age is reached, perhaps after a long period of conservative treatment has proved unsuccessful.

The sex distribution—31 girls and 3 boys—cannot be regarded as representative of the incidence of dysplasia but largely reflects the preponderance of female cases of pyelonephritis, especially where it is accompanied by ureteroceles and ectopic ureters; this is in contrast to the male predominance in the case of neonatal pyelonephritis.[14] Of the 34 patients, 30 had infection of the urinary tract. Four patients had no history of this infection and presented no such signs on examination. Of these, two—the autopsy cases—were newborn.

Vesicoureteral reflux was found in 7 out of 17 patients examined. In all seven cases there was infection of the urinary tract.

No unilateral predominance was found in the clinical series (Tables 1 to 3).

Histologic Criteria.—In the diagnosis of renal dysplasia the following histologic features may be considered.

Primitive Ducts: These structures, which were usually found, singly or in groups, in the medullary part of the kidney and near the pelvis, were lined with cuboidal epithelium and had concentric rings of connective tissue that seemed to contain collagen but no elastin. Occasionally, fibers of smooth muscle were also found. These ducts are thought

TABLE 1.—*Group 1, Renal Dysplasia*

Specimen	Age	Sex	Side	Oper.*	Ducts	Med. Dyspl.	Primitive		Cart.	Lymph. Tissue	Inflam. Cells
							Glom.	Conv. Tub.			
1	6 yr.	F	R	N	+	−	−	+	−	−	+
2	4 yr.	F	R	N	+	+	+	+	−	+	+
3	4 yr.	F	L	H	+	+	−	−	−	+	+
4	4 yr.	F	R	H	+	+	+	+	−	+	+
5	4 yr.	F	L	H	+	+	+	+	−	+	+
6	4 yr.	F	R	H	+	+	−	−	−	+	+
7	4 mo.	F	R	H	+	+	+	+	−	+	+
8	8 yr.	F	L	H	+	+	+	+	−	−	+
9	1 yr.	F	L	H	+	+	+	+	−	+	+
10	9 yr.	F	R	H	+	+	−	+	−	+	+
11	1 yr.	F	L	N	+	+	+	+	+	+	+
12	5 yr.	F	L	H	+	+	+	+	−	+	+
13	7 days	M	R	A	+	+	+	+	+	−	−
14	1 day	M	R	A	+	+	+	+	−	−	−
15	2 yr.	F	L	H	−	+	−	+	+	−	+

* N indicates nephrectomy; H, heminephrectomy; A, autopsy.

TABLE 2.—*Group 2, Apparent Dysplasia*

Specimen	Age	Sex	Side	Oper.*	Ductules	Med. Dyspl.	Primitive		Lymphoid Tissue	Inflam. Cells
							Glom.	Conv. Tub.		
16	4 yr.	F	L	N	+	+	+	+?	+	+
17	3 yr.	F	R	H	+	+	+	+	+	+
18	10 yr.	F	R	H	+	+	—	+	+	+
19	11 yr.	F	R	N	+	—	—	+?	+	+
20	2 yr.	F	L	H	+	+	+	+	+	+
21	10 mo.	F	R	H	+	+	+	+	+	+
22	8 yr.	F	L	H	+	+	—	+	+	+
23	6 mo.	F	L	H	+	+	+	+	+	+
24	7 yr.	F	R	N	+	+	—	—	+	+
25	2 yr.	F	L	H	+	+	+	+	+	+
26	5 yr.	F	R	H	+	+	+	+	+	+
27	6 yr.	F	L	H	+	+	—	—	+	+
28	6 mo.	F	R	H	+	+	+	—	+	+
29	1 yr.	F	L	H	+	+	—	—	+	+
30	5 yr.	F	R	N	+	+	—	—	+	+
31	5 yr.	F	R	H	+	+	—	—	+	+

* N indicates nephrectomy; H, heminephrectomy.

to be remnants of the mesonephric (Wolffian) duct.[15,16] They closely resemble the aberrant ductules of the Fallopian tube.

The primitive ducts varied in size, the largest being found in grossly abnormal specimens with poor cortical differentiation. In less severely malformed specimens fairly large primitive ducts were found in the peripelvic tissue adjacent to the renal parenchyma.

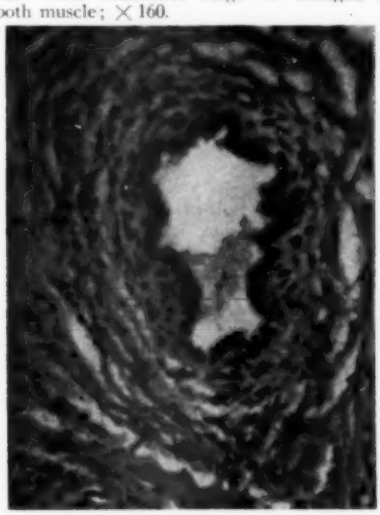
Medullary Dysplasia: This term refers to the condition in which the medulla contained very few collecting tubules and papillary ducts in a noncicatricial stroma rich in collagenous fibers and having no inflammatory cells. Some of these ducts were very similar to the primitive ducts described above; there were concentric rings of connective tissue, occasionally with smooth muscle. In some cases most of the medulla was composed of such

TABLE 3.—*Group 3, No Apparent Renal Dysplasia*

Specimen	Age	Sex	Side	Oper.*	Ductules	Med. Dyspl.	Primitive		Lymphoid Tissue	Inflam. Cells
							Glom.	Conv. Tub.		
32	7 yr.	F	L	H	+?	—	—	—	+	+
33	4 yr.	F	L	N	+?	—	—	—	+	+
34	4 mo.	M	L	N	—	—	+	—	+	+

* H indicates heminephrectomy; N, nephrectomy.

Fig. 2 (Case 8).—Primitive ducts in peripelvic tissue. Note concentric rings of collagen and smooth muscle; × 160.



ducts; only rarely were there ducts in apparently normal tissue.

Ductules: The cortex, the medulla, and the peripelvic tissue often contained ducts that were smaller than the primitive ducts but apparently similar in structure, though with no smooth muscle. The cuboidal epithelium was surrounded by a thin layer of concentric connective tissue rings.[17]

Cartilage: The presence of hyaline cartilage in the kidney is regarded as a congenital malformation.[18]

Primitive Glomeruli: Glomeruli were classed as primitive when the first three stages of their development—the S-form, the nonvascular bud, or the retained fetal lobulation—described by Macdonald and Emery[19] were observed. Abnormal primitive glomeruli, when present, were encountered next to ductules. This suggests a relationship between these structures, a possibility that has been examined more closely.[17]

Primitive Convoluted Tubules: This was a feature in which the epithelium of the tubules was abnormally high and the tubules followed an ir-

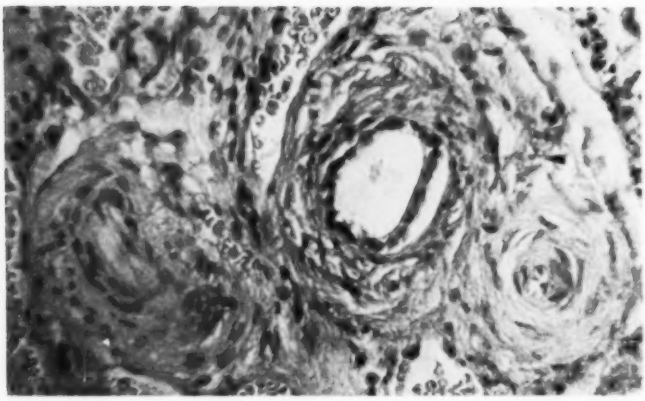


Fig. 3 (Case 19).—Cortical ductule between arterioles. Note cuboidal epithelium and concentric rings of collagen. Reduced about 15% from mag. ×320.

regular course. When present in the cortex corticis, they were adjacent to primitive glomeruli. They resembled fetal tubules.

Lymphoid Tissue: The presence of lymph follicles with germinal centers was recorded whenever they occurred in the renal parenchyma, as it has been suggested that this feature may be a malformation and thus does not necessarily imply infection.[9]

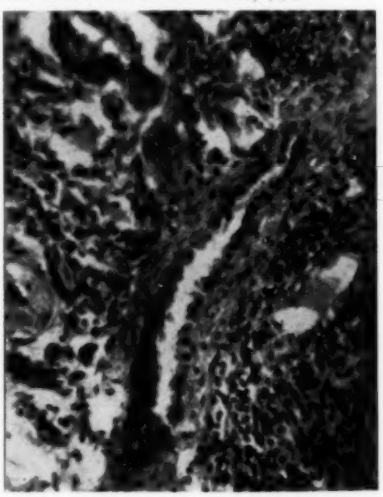
Of these histologic features only two—*primitive ducts* and *cartilage*—were regarded as indisputable evidence of embryonic maldevelopment, a kidney containing either or both of them automatically being labeled as dysplastic.

The other features defined were looked upon as providing an insufficiently sound basis for the classification of the specimen, whether they occurred alone or in combination. The arbitrary term *medullary dysplasia* covered changes that may have been due to secondary atrophy, such as ischemia and inflammation that resulted in fibrosis. This may have been the case even where inflammatory cells and pathologically changed vessels were absent. The *ductules* described may have resulted from renal atrophy and subsequent displacement of changed tubules—into the medulla or the peripelvic tissue, for instance. *Primitive glomeruli* and *convoluted tubules* were difficult to establish as primitive; in the case of the tubules, regeneration following damage to tubules could not be ruled out. Further, as primitive glomeruli are a difficult feature to evaluate in any kidney,[10] their presence was not considered of diagnostic value. The presence of *lymphoid tissue* was of interest since some cases presented no symptoms or signs of infection, but it was not regarded as of significance in classifying the specimens.

Pyelonephritis: In this paper pyelonephritis denotes interstitial infiltration of lymphocytes and plasma cells in the renal parenchyma and of inflammatory cells in the renal pelvis. The parenchymatous lesions were usually situated in the cortex and displayed a focal disposition; they were associated with groups of partly, or completely, scarred glomeruli and atrophic tubules, which were occasionally reminiscent of goiter.

The renal tissue removed from the 34 patients was fixed in formalin. Between two and five blocks were embedded in paraffin, and sections were cut. Most of them were stained by Van Gieson's method, a small number being counterstained with Weigert's elastin. A few specimens were treated with hematoxylin and eosin. No weights are given, since the material was not selected on the basis of the size of the specimen but was a consecutive series of kidney specimens, most of them from heminephrectomies done on infants and children.

Fig. 4 (Case 8).—Cortical ductule in longitudinal section. Interstitial inflammation; ×160.



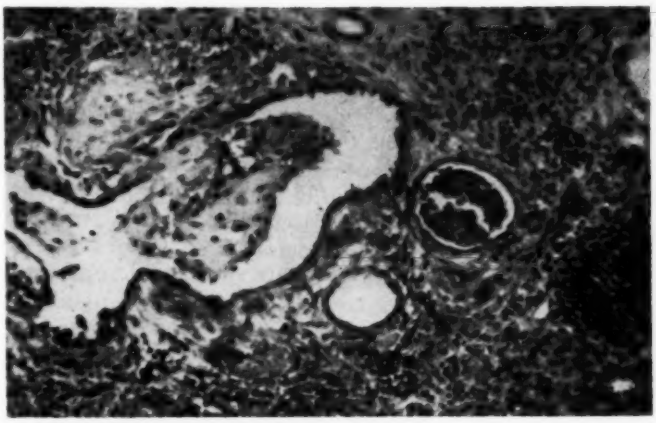


Fig. 5 (Case 21).—Abnormal primitive tubule in cortex with inflammatory change. Reduced 20% from mag. × 160.

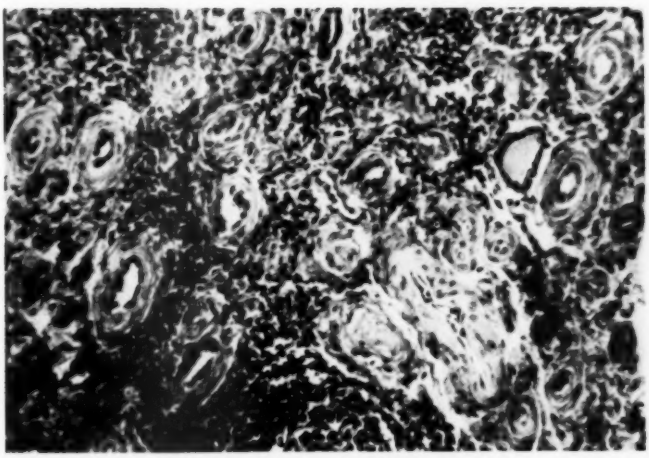


Fig. 6 (Case 16).—Renal dysplasia with numerous corticomedullary ductules. Lymphoid tissue in lower center. Interstitial inflammation, atrophic tubule to the right. Reduced 15% from mag. × 160.

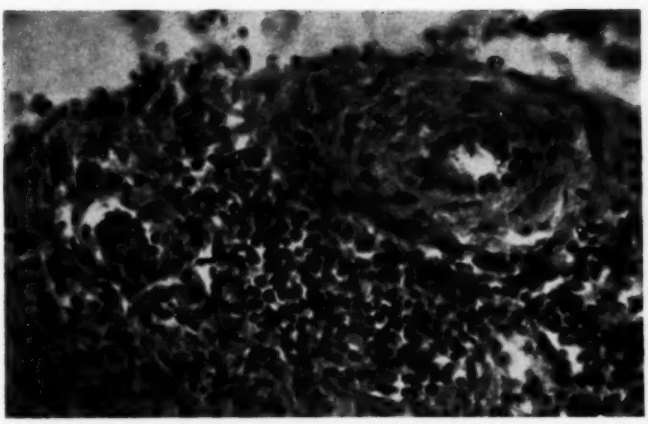


Fig. 7 (Case 16).—Subcapsular ductule to the right and primitive abnormal glomerulus to the left in area of inflammation. Reduced 20% from mag. × 320.

The sections were studied without clinical bias. Classification according to the above criteria gave three provisional groups: (1) *renal dysplasia*, (2) *apparent renal dysplasia*, and (3) *no apparent renal dysplasia*.

Results

Group 1: Renal Dysplasia (15 Specimens).—This group comprised all the specimens in which primitive ducts or cartilage were found. They could be arranged to represent a gradual course of evolution, starting with the most primitive and cystic structure containing primitive ducts and one or two isolated tubules next to primitive glomeruli in loose connective tissue, with cartilage; as an intermediate stage there was a moderately condensed kidney with a dysplastic medulla containing primitive ducts and a narrow cortex rich in vessels; finally, there were very slightly reduced specimens in which there were primitive ducts and occasional cortical scars accompanied by ductules.

The other histologic features are summarized in Table 1.

In addition to signs of maldevelopment there were inflammatory changes of varying degrees of severity. In 13 cases lymphocytes and plasma cells were found in the wall of the ureter, the pelvic mucosa, the medulla, and the cortex. The two autopsy specimens displayed no inflammatory cells. Two cases in which no clinical signs of infection had been observed presented histologic evidence of inflammation. In four specimens there were mineral salt deposits in the lumens of cortical tubules.

Group 2: Apparent Renal Dysplasia (16 Specimens).—The specimens assigned to this group presented ductules in the cortex and medullary dysplasia. Twelve specimens were removed from the upper pole of kidneys with double ureters, and four were recovered at nephrectomy (Table 2). The majority of the specimens consisted of hydronephrotic sacs. Of the four kidneys removed, two showed hydronephrosis (Specimens 16 and 30).

The specimens in this group generally displayed rather prominent cortical fibrosis and scarring concomitant with severe hydronephrosis; this gave a sac-like appearance to the specimen, a feature that rendered the evaluation of possible dysplastic elements more difficult. Close examination of sections from the entire material revealed two recurrent features—apart from fibrotic scarring and inflammatory processes—namely, the above medullary picture and the presence in the cortex of tiny round ductules having more or less prominent connective tissue rings that stained like collagen. All specimens having both ductules and a paucity of collecting tubules in the medulla were assigned to a single group.

There were no structural differences between the heminephrectomy specimens with double ureters and the whole kidneys without this feature. It may be inferred that ureteral duplication is not a prerequisite for medullary dysplasia or for the occurrence of cortical ductules.

As in Group 1, scars were often encountered, these being infiltrated with lymphocytes and occasional plasma cells. Ductules were found in the scars. In some cases they were situated not only beneath the capsule but also in the middle of the cortex, at the corticomedullary junction, and in the medulla proper—as in Specimen 17.

The structures described may well have a developmental origin, although this is at present difficult to prove.

Group 3: No Apparent Renal Dysplasia (3 Specimens).—Two specimens were removed by nephrectomy, and one, by resection (Table 3). The two first presented hydronephrosis with single ureters (Specimens 33 and 34). The heminephrectomy specimen (Specimen 32) had two ureters, and the renal parenchyma was moderately reduced due to hydronephrosis.

The sections showed a reduced renal parenchyma with large areas of chronic pyelonephritis. There was no conclusive evidence of medullary dysplasia. Where

the medulla was present, it was scarred and evidenced inflammatory cells.

In the cortex of two specimens, and usually associated with cortical scars, there were occasional structures bearing some resemblance to ductules. Owing to the extensive inflammatory changes, the identity of these structures could not be definitely established. The third specimen (Specimen 34)—from a male—did not display any ductules in any of several sections.

Comment

It is generally agreed that the histologic differentiation of dysplasia and secondary renal atrophy usually presents considerable difficulty. Marshall [1] found fetal structures in pyelonephritic kidneys from infants and children and concluded that focal dysplasia is commonly the underlying cause of pyelonephritis in childhood. Moreover, he recently [2] showed the presence of maldeveloped tissue elements such as primitive glomeruli and tubules in the subcapsular area of renal scars He compared these primitive tubules to collecting tubules and stressed the possibility that a vascular abnormality is probably basically responsible for the persistence of fetal tissue in these areas of subsequent scarring.

The analysis of a consecutive series of 34 kidney resections and nephrectomies performed on infants and children resulted in the definite diagnosis of dysplasia in 15 cases and a probable diagnosis in 16. Three specimens revealed no definite signs of maldevelopment, although in two there were primitive ductules of the cortex.

Nonobstructive pyelonephritis was present in seven instances, which were histologically indistinguishable from specimens with a possible obstructive origin of the pyelonephritis. In the Group 2 specimens where dysplasia was suspected, primitive ductules were encountered in all cortical foci of inflammation; moreover, the medulla showed a paucity of collecting tubules, some of which had thick serous coats of collagen and, occasionally, of smooth muscle. The ducts and ductules had the same appearance as the mesonephric tubules and their remnants—for instance, the aberrant ductules of the Fallopian tube.

Before the ductules can be accepted as evidence of maldevelopment the possibility of an inflammatory origin must be ruled out. Despite the fact that they were present in inflamed tissue, the ductules seemed never to display inflammatory impairment. Their cuboidal or columnar epithelial lining was intact, and there were no inflammatory cells in the walls. The concentric rings were distinct and unscarred. They were discrete, although several could be found in the same medium-power field. Except for their size they were closely similar to the large primitive ducts of the Group 1 specimens. The ductules never displayed the goiter-like formation of the atrophic tubules in pyelonephritis. A special study of their origin and course has been made.[17] Suffice it to say that there is convincing evidence that the ductules are of congenital origin. Of the 34 consecutive kidney specimens 33 would then be classed as examples of renal dysplasia.

Twenty-nine of the thirty-one specimens revealed chronic pyelonephritis, and in many cases the inflammatory changes were so severe as to conceal the dysplasia. The inflammatory changes were concomitant with the dysplastic ones, and the fact that two patients presenting renal dysplasia with no inflammation died at ages of 1 and 7 days suggests that the dysplastic changes are primary to the inflammatory changes rather than the reverse. Further, in two cases (Specimens 4 and 12) there were histologic signs of inflammation but never was there any clinical evidence of infection. It is clear that it is by no means an easy matter to differentiate between pyelonephritis and subclinical infection of the urinary tract.

The structures that have here been called ductules have apparently been noted by previous investigators. Marshall [1,2] describes "primitive tubules," a term that he seems to apply rather widely to primitive con-

voluted tubules with high columnar epithelium and to structures that are similar, and probably identical with our ductules (Marshall,[1] Fig. 77, Case 12). Two of Allen's [20] plates portray renal dysplasia with primitive nephrons, some of which bear a close resemblance to the ductules (Plates 40 and 41, pp. 97 and 99). Other authors, among them Johnson and Anderson,[21] discuss the possibility of malignant renal tumors originating from developmental remnants of primitive tubules. Cicchino et al.[22] have called attention to the resemblance between primitive tubules and the epididymal ducts in renal dysplasia.

The cause of the renal dysplasia is obscure. As for ischemia,[1,2] the excess vascularization of the dysplastic areas and the malformed walls of some vessels are difficult to evaluate quantitatively in kidneys that are severely impaired by inflammatory processes, with consequent atrophy.

Several specimens in Groups 1 and 2 were associated with double ureters. In these cases other evidence of ureteric maldevelopment in the form of primitive ducts might be expected. It is generally accepted that the ureteric bud acts as an organizer of the metanephrogenic tissue,[23,25] and one would expect to find malformation of this tissue in cases of ureteric maldevelopment. If morphologic similarity can be accepted as proof of a common origin, the ductules found in the cortex might be supposed to be derived from the ureteric bud. This would lend further support to the view that abnormal ureteric budding is an essential factor in renal dysplasia.

The case material provides no indication of the pathways of the concomitant pyelonephritis. Remains of fetal tissue in a fully developed organ are considered to be particularly vulnerable to infection, but there is no reason for assuming any particular route of infection, which might be hematogenous, lymphogenous, or canalicular.

Whatever the paths by which the infection reaches the cortical areas concerned, the results of this study support the hypothesis that they are particularly vulnerable and constitute an important predisposing factor in pyelonephritis in infancy and childhood.

Summary

Clinical, roentgenologic, and histologic examinations were performed on 34 children, 30 of whom presented infections of the urinary tract. Eight were under 1 year of age, and twenty-six of them, between 1 and 11 years. Malformations of the urinary tract were common, 16 patients having ureterocele and 9, ectopic ureters. This is an inevitable feature of any pediatric case material, since cases with no such malformations rarely undergo operation. Thirty-one girls and three boys were encountered, a sex distribution due to the female predominance in pyelonephritis and ureteric malformations rather than in renal dysplasia. No left preponderance was found.

The specimens were grouped in three categories: 1. *Dysplastic* (15 specimens)—malformed kidneys with numerous primitive ducts resembling the mesonephric duct. Thirteen presented pyelonephritis. 2. *Suspected dysplasia* (16 specimens)—kidneys mostly hydronephrotic, with few medullary ducts ("medullary dysplasia") and primitive cortical "ductules," resembling mesonephric ductules. All specimens displayed severe pyelonephritis. 3. *No obvious renal dysplasia* (3 specimens)—structures resembling ductules were present in two; no medullary dysplasia. Striking inflammatory changes.

The microscopic findings suggest abnormal budding of the ureter as the underlying cause of the dysplasia. The cortical ductules described may be evidence of renal dysplasia—probably abnormal proliferation of ureteric buds. Concomitant inflammation where the patient survives for more than seven days indicates that renal dysplasia is an important predisposing element in pyelonephritis in infancy and childhood.

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REFERENCES

1. Marshall, A. G.: The Persistence of Fœtal Structures in Pyelonephritic Kidneys, Brit. J. Surg. 41:38-50, 1953.
2. Marshall, A. G.: Scars of the Renal Cortex, J. Path. & Bact. 71:95-109, 1956.
3. Ekström, T.: Renal Hypoplasia: A Clinical Study of 179 Cases, Acta chir. scandinav., Supp. 203, 1955.
4. Ritter, J. S., and Kramer, S. E.: Hypoplastic Kidney and Atrophic Pyelonephritic Kidney, J. Urol. 63:48-57, 1950.
5. Emmett, J. L.; Alvarez-Ierena, J. J. and McDonald, J. R.: Atrophic Pyelonephritis Versus Congenital Renal Hypoplasia, J. A. M. A. 148: 1470-1477, 1952.
6. Harbitz, F.: Ureterabnormiteten; Nephrolithiasis, Ztschr. urol. Chir. 19:70-71, 1925.
7. Ainsworth, T.: Unilateral Renal Agenesis, South. M. J. 29:619-622, 1936.
8. Urology, edited by M. Campbell, Philadelphia, W. B. Saunders Company, 1954.
9. Ask-Upmark, E.: Über juvenile maligne Nephrosklerose und ihr Verhältnis zu Störungen in der Nierenentwicklung, Acta path. et microbiol. scandinav. 6:383-442, 1929.
10. Fahr, T.: Über pyelonephritische Schrumpfniere und hypogenetische Nephritis, Arch. path. Anat. 301:140-185, 1938.
11. Heritage, K.; Scarff, R. W.; Evans, H., and Wright, A. D.: Discussion on the Relationship of Hypertension to Renal Disease, Proc. Roy. Soc. Med. 36:551-564, 1943.
12. Burkland, C. E.: Clinical Considerations in Aplasia, Hypoplasia and Atrophy of the Kidney, J. Urol. 71:1-13, 1954.
13. Kjellberg, S. R.; Ericsson, N. O., and Rudhe, U.: The Lower Urinary Tract in Childhood, Chicago, The Year Book Publishers, Inc., and Stockholm, Almqvist & Wiksell, 1957.
14. Porter, K. A., and Giles, H. McC.: A Pathologic Study of 5 Cases of Pyelonephritis in the Newborn, Arch. Dis. Childhood 31:303-309, 1956.
15. Eisendraht, D. N.: Clinical Importance of Congenital Renal Hypoplasia, J. Urol. 33:331-355, 1935.
16. Ericsson, N. O.: Ectopic Ureterocele in Infants and Children: Clinical Study, Acta chir. scandinav. Supp. 197, pp. 1-92, 1954.
17. Ericsson, N. O., and Ivemark, B. I.: Renal Dysplasia in Infants and Children: Part II. Primitive Ductules and Abnormal Glomeruli, A. M. A. Arch. Path., this issue, p. 264.
18. Bigler, J. A., and Killingsworth, W. P.: Cartilage in the Kidney, Arch. Path. 47:487-493, 1949.
19. Macdonald, M. S., and Emery, J. L.: Personal communication to the authors, 1957.
20. Allen, A. C.: The Kidney: Medical and Surgical Diseases, New York, Grune & Stratton, Inc. 1951.
21. Johnson, F. R., and Anderson, J. C.: Developmental Remnants in an Adult Human Kidney, J. Path. & Bact. 66:39-46, 1953.
22. Cicchino, F. E.; Yates, T. M., and Chappell, B. S.: Vestigial Epididymal Structures in Unilateral Renal Aplasia, J. Urol. 66:667-672, 1951.
23. Huber, G. C.: On the Development and Shape of Uriniferous Tubules of Certain of the Higher Mammals, Am. J. Anat. (Supp.) 4:1-98, 1904-1905.
24. Fischel, A.: Die Bedeutung der Entwicklungsmechanischen Forschung für die Embryologie und Pathologie des Menschen, Vortr. u. Aufs. Entwmech. Org. 16:1-69, 1912.
25. Boyden, E. A.: Congenital Absence of the Kidney: An Interpretation Based on a 10-Mm. Human Embryo Exhibiting Unilateral Renal Agenesis, Anat. Rec. 52:325-349, 1932.

Renal Dysplasia and Pyelonephritis in Infants and Children

Part II. Primitive Ductules and Abnormal Glomeruli

NILS OLOF ERICSSON, M.D., and BIÖRN I. IVEMARK, M.D., Stockholm, Sweden

Renal dysplasia denotes the presence in the renal parenchyma of cysts, cartilage, and retained fetal tissue elements, such as primitive ducts [1,2] and tubules.[3,4] It is generally considered that in the dwarfed kidney pyelonephritis may often be so severe that dysplasia will escape detection even by histologic examination.[5] The prevalent opinion, however, is that renal dysplasia predisposes a kidney to pyelonephritis.[3,6]

In the preceding paper [2] the presence of renal dysplasia was established in 15 out of 34 kidney specimens from infants and children, and in the case of another 16 specimens showing pyelonephritis the presence of renal dysplasia was highly probable. In the specimens comprising the latter group, structures designated as "ductules" were described. Though the presence of these ductules was not then considered as definite evidence of dysplasia, it was pointed out that abnormal and primitive glomeruli were often encountered adjacent to the cortical ductules. It is the purpose of this paper to point to a possible relationship between the ductules and the abnormal glomeruli and to discuss the origin of the ductules.

Submitted for publication March 3, 1958.

From the Departments of Pediatrics and Pathology, Karolinska Sjukhuset.

Experimental Data

Three kidney specimens (Cases 16, 17, and 20 of the preceding study [2]) were serially sectioned.

Specimen A (Case 16 [2]).—This was a dwarfed left kidney removed by nephrectomy from a 4-year-old girl with recurrent infection of the urinary tract. There were bilateral ureteric duplication and an ectopic ureterocele originating from the left upper ureter. Blocks from the formalin-fixed specimen were embedded in paraffin, serially sectioned, and stained with Van Gieson's connective tissue stain.

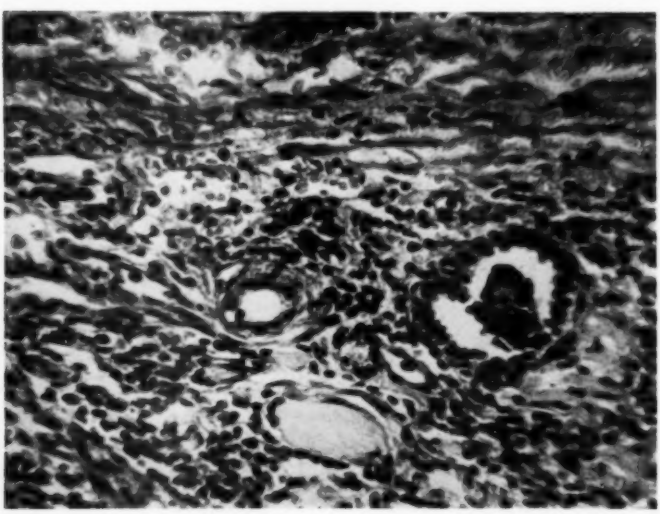

Fig. 1.—Subcapsular area of inflammation containing primitive abnormal glomerulus to the right and ductule to the left. Note atrophic tubule below. Reduced about 5% from mag. ×320.

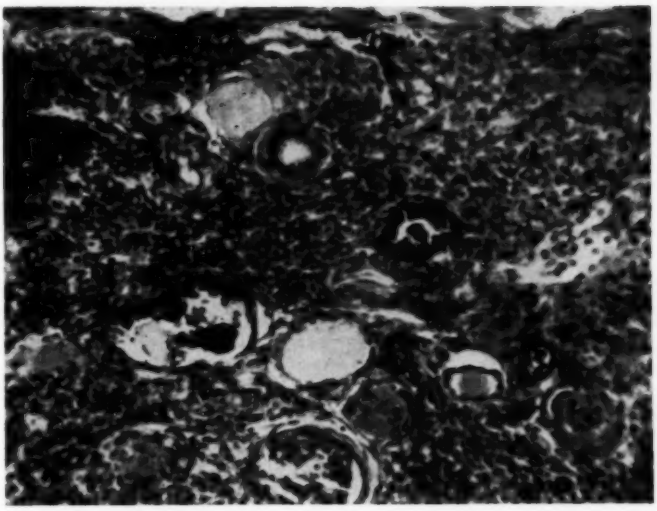

Fig. 2.—Area of inflammation displaying ductule in top center, two abnormal primitive glomeruli below, and hyalinized ones in lower part of picture. Atrophic tubule adjacent to ductule. Reduced about 5% from mag. × 160.

The histologic sections showed areas of severe chronic pyelonephritis with cortical scars and numerous ductules, these occasionally being associated with abnormal primitive glomeruli.

Specimen B (Case 17[5]).—This was a walnut-sized upper pole of a right kidney removed by resection from a 3-year-old girl with recurrent infections of the urinary tract. The resected portion included dilated calyces and a reduced renal parenchyma. Blocks of the formalin-fixed specimen were embedded in paraffin and serially sectioned.

Histologic examination disclosed similar changes to those found in Specimen A—marked chronic pyelonephritis and many cortical ductules.

Specimen C (Case 29[2]).—This was the upper third of the left kidney from a 15-month-old girl with infection of the urinary tract and ectopic ureterocele of the left upper ureter. The specimen measured 3 by 4 by 2 cm. Blocks of the formalin-fixed specimen were embedded in paraffin, and serial sections cut.

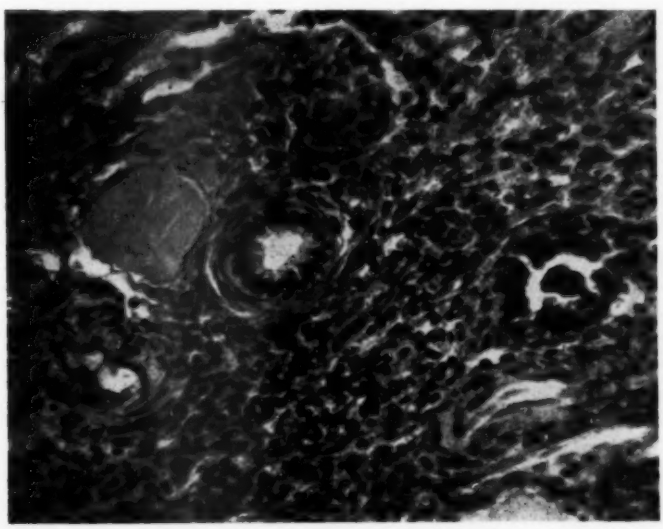

Fig. 3.—Detail of Figure 2, showing ductule, atrophic tubule, and primitive glomerulus. Reduced about 5% from mag. × 320.

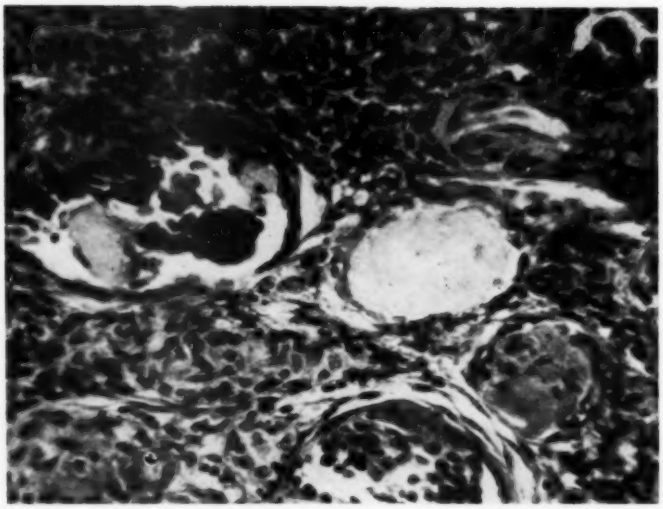

Fig. 4.—Detail of Figure 2, showing primitive glomerulus with calcification, atrophic tubule with cast, and hyalinized glomeruli. Reduced about 5% from mag. × 320.

The microscope revealed patches of marked chronic pyelonephritis with scarring and numerous cortical ductules, some of them adjacent to abnormal primitive glomeruli.

Results

Study of the serial sections of all the specimens was centered on the course of the ductules, especially those adjacent to abnormal glomeruli. Ductules were found in the peripelvic tissue, perivascularily, throughout the medulla, and penetrating the cortex to the capsule. Some ductules could be traced from the juxtacortical part of the medulla and through the cortex to beneath the fibrous renal capsule, where they ended blindly. In this region they often made a bend and followed a course parallel to the renal surface under the capsule.

The ductules were never found to join uriniferous convoluted tubules. Nevertheless, their course to some degree resembled that of collecting tubules, and they seemed to correspond to the medullary rays of the normal kidney. In the subcapsular area occasional abnormal primitive glomeruli were found adjacent to a ductule. There was no communication between ductules and glomeruli.

The primitive and abnormal glomeruli showed a dense parietal layer of high columnar epithelium with dark nuclei. The parietal basement membrane was usually not thickened. The glomerular tuft was lobulated, small, and bizarre, with a layer of dark-staining high columnar epithelium. Some glomerular spaces were dilated and contained hyaline casts. A few tufts showed deposition of mineral salts. In other instances the glomerular tuft was partly hyalinized. Groups of completely hyalinized glomeruli were often found adjacent to ductules.

The abnormal glomeruli were either isolated or connected to dilated convoluted tubules that had an atrophic flattened epithelial lining and eosinophilic casts. There was no concentric peritubular fibrosis of the proximal convoluted tubules.

The interstitial tissue displayed dense infiltration of lymphocytes and plasma cells. Areas of lymphoid tissue were found—occasionally adjacent to ductules.

The glomeruli of Specimen C were more primitive in appearance than of Specimens A and B. In addition to glomeruli with partly scarred tufts, and groups of completely scarred glomeruli that were features of Specimens A and B, the sections of Speci-

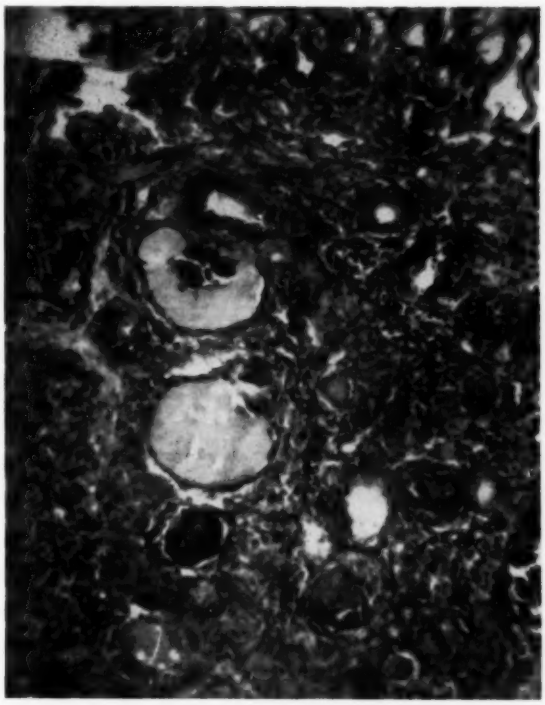

Fig. 5.—Abnormal subcapsular glomeruli, the upper with small tuft and adjacent to ductule. Serial sections showed the latter to end blindly near glomerulus; × 160.

men C contained abnormal primitive glomeruli located adjacent to ductules and having no scars at all. The difference in the histologic picture was ascribed to the considerably lower age of the patient supplying Specimen C.

Comment

The course of the ductules was similar to that of the medullary rays of the normal kidney, from which it may be inferred that the ductules are abnormal straight collecting tubules. The periductal layer of concentric connective tissue was a characteristic feature, which rendered the ductules conspicuous in sections stained by Van Gieson's method. They did not make the connections of typical straight collecting tubules but were found to pass by the uriniferous tubules to the subcapsular area, leaving the corresponding proximal part of the nephron with no normal outlet. The primitive and abnormal glomeruli were connected to atrophic blind tubular segments. The attached glomeruli were maldeveloped, probably owing to a combination of atrophy of disuse and arrested development. The vascular supply of the glomerular tuft presumably decreased and resulted in partial or complete hyalinization.

It should be noted that the described absence of union gave rise to atrophy rather than to gross cysts. This is contrary to the theories on the pathogenesis of polycystic kidneys.[7] Moreover, the dilatation of Bowman's space never approaches the size of glomerular cysts.[8,9] Microdissection has, furthermore, shown these to communicate with the renal pelvis.[9]

The course of the ductules, together with the fact that they occur apart from inflammation, would suggest that they represent evidence of dysplasia rather than that they derive from an inflammatory process. This concurs with the opinion of Obiditsch-Mayer,[10] who found similar ductules in 13

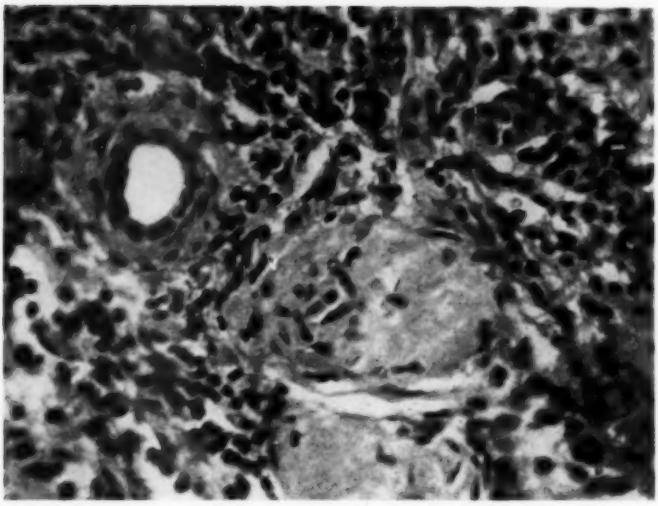

Fig. 6.—Ductule and hyalinized glomerulus—interpreted as the final stage of glomerular change owing to lack of union between the two parts of the nephron. Note interstitial inflammation. Reduced about 5% from mag. × 320.

dysplastic kidneys. He demonstrated neurofibrils in their outer coat and held that there is a neurovascular component in the pathogenesis of renal dysplasia.

Remnants of fetal tissue in otherwise normal organs are regarded as being particularly vulnerable to infection. Likewise, ischemia tends to favor infection. The dysplastic areas of the kidney specimens would thus be more vulnerable to infection than the surrounding nondysplastic renal tissue. The findings might also be interpreted as favoring a hematogenous path of pyelonephritic infection. The presence of inflammatory cells within and around the dysplastic areas might thus be explained on a dysontogenetic basis, the dysplastic renal tissue being the underlying cause of the pyelonephritis.

The specimens contained grossly abnormal ureters with duplication. Though their simultaneous occurrence is a striking phenomenon, the apparently abnormal intrarenal ureteric budding and the ureteric duplication cannot, on the evidence of the findings, be considered as having a causal connection. Purely by way of speculation, it might be suggested that the association of the supernumerary upper ureter with the dysplastic part of the kidney was due either to a second metanephric bud or to an abnormally retained true Wolffian duct.

Certain it is, however, that for some obscure reason the ureteric bud went astray during renal organogenesis and resulted in ureteric duplication, abnormal renal pelvis (hydronephrosis), and abnormal organization of the metanephrogenic blastema. It is known that the ureter is necessary for normal differentiation of the metanephrogenic tissue,[11] and it seems likely from the findings of this study that abnormal ureteric development may result in renal dysplasia.

Summary and Conclusions

Three specimens previously reported as examples of presumably dysplastic kidneys were examined by serial sectioning. Severe chronic pyelonephritis was revealed, the pyelonephritic areas containing primitive cortical ductules. The ductules were occasionally adjacent to abnormal primitive glomeruli. The ductules were shown to originate in the medulla and ended blindly in the cortex beneath the capsule, without joining the uriniferous tubules. The primitive and abnormal glomeruli were isolated or connected to atrophic dilated proximal uriniferous tubules with no manifest outlet.

It is concluded that (1) the ductules described represent maldeveloped collecting tubules that had their origin in abnormal ureteric development; (2) absence of a union between the ureteric and metanephric parts of the nephron may have been the reason for the abnormal evolution of the glomeruli of the blind segments; (3) when ductules are found in a kidney it should be regarded as dysplastic; (4) as the dysplastic areas displayed chronic inflammatory changes, renal dysplasia—local or general—is presumably one predisposing element in pyelonephritis.

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REFERENCES

1. Baggenstoss, A. H.: Symposium on Diseases of Kidney: Congenital Anomalies of the Kidney, M. Clin. North America 35:987-1004, 1951.

2. Ericsson, N. O., and Ivemark, B. I.: Renal Dysplasia and Pyelonephritis in Infants and Children: Part I., A. M. A. Arch. Path., this issue, p. 255.

3. Marshall, A. G.: The Persistence of Fœtal Structures in Pyelonephritic Kidneys, Brit. J. Surg. 41:38-50, 1953.

4. Marshall, A. G.: Scars of the Renal Cortex, J. Path. & Bact. 71:95-109, 1956.

5. Emmett, J. L.; Alvarez-Ierena, J. J., and McDonald, J. R.: Atrophic Pyelonephritis Versus Congenital Renal Hypoplasia, J. A. M. A. 148:1470-1477, 1952.

6. Porter, K. A., and Giles, H. McC.: A Pathological Study of 5 Cases of Pyelonephritis in the Newborn, Arch. Dis. Childhood 31:303-309, 1956.

7. Lambert, P. P.: Polycystic Disease of Kidney: Review, Arch. Path. 44:34-58, 1947.

8. Ivemark, B. I.; Oldfelt, V., and Zetterström, R.: Familial Dysplasia of Kidneys, Liver and Pancreas in Infancy, Acta pædiat., to be published.

9. Bialestock, D.: The Morphogenesis of Renal Cysts in the Stillborn Studied by Micro-Dissection Technique, J. Path. & Bact. 71:51-59, 1956.

10. Obiditsch-Mayer, I.: Ein Beitrag zur Frage der Nierenhypoplasie, Verhandl. deutsch. Gesellsch. Path. 40:187-191, 1956.

11. Boyden, E. A.: Congenital Absence of the Kidney: An Interpretation Based on a 10-Mm. Human Embryo Exhibiting Unilateral Renal Agenesis, Anat. Rec. 52:325-349, 1932.

Skeletal Lesions Produced in Rats by Feeding Beta-Mercaptoethylamine

Comparison with Lesions Due to Beta-Aminopropionitrile (Toxic Lathyrus Factor)

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Beta-mercaptoethylamine (Becaptan; $NH_2CH_2CH_2SH$) and certain other sulfhydryl compounds have been found to protect the organism against the injurious effect of ionizing radiations. Bacq et al.[1] reported that injection of 3 mg. of β-mercaptoethylamine one to three minutes before exposure of mice to a lethal dose of radiation resulted in a permanent survival of 97% of the experimental animals. They did not find any obvious toxicity in their animals to preclude the use of the drug for clinical purposes, and a single intravenous injection of 200 mg. in cancerous patients treated with ionizing radiations completely eliminated within 24 hours all symptoms of radiation sickness.

On the other hand, Dasler,[2] by feeding rats with β-mercaptoethylamine, noted skeletal deformities at the end of four weeks which appeared similar to those produced by β-aminopropionitrile. This report was based on the gross examination of the femur and mandible in these animals, but apparently no detailed pathological studies were made.

This subject is therefore of more than academic interest, since β-mercaptoethylamine appears to have more than one type of biological action. This investigation was done to study the pathological effects of β-mercaptoethylamine, when administered both parenterally and orally, on the skeletal system.

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Materials and Methods

Fifty Wistar rats, both male and female, 3 weeks old, freshly weaned, and weighing about 30 gm. were divided into three groups:

Group I: Fifteen rats were fed a standard diet (Ration No. 10, supplied by the Buckerfield Feed Company).

Group II: Fifteen rats were fed the standard diet and received intraperitoneal injection of 20 mg. of β-mercaptoethylamine once a day.

Group III: Twenty rats were fed *continuously* the standard diet, with which β-mercaptoethylamine hydrochloride was mixed so that it made a concentration of 0.5% in the diet.

All animals were weighed once a week throughout the experiment.

At least two animals from each group were killed at about the third, sixth, and eighth weeks. Most of the animals of Group III died between the 10th and 12th weeks. There were no deaths in the other two groups. The surviving animals were therefore killed between the 10th and 12th weeks.

X-rays were taken when the animals were either killed or died and a gross pathological examination was made. The knee joint and shoulder joint were selected for histological study. The tissues were fixed in 10% formol alcohol and decalcified with 1 N HCl. Paraffin blocks were prepared, and serial sections from each block were stained as follows: Hematoxylin and eosin, 1% aqueous toluidine blue, periodic acid Schiff (McManus method). Undecalcified tissue of the same site was similarly prepared, and the sections, stained for calcium by the von Kossa method.

Results

Gross Study.—The animals in Groups I and II, i. e., the normal controls and those

that received mercaptoethylamine by injections, appeared healthy, showed a normal gain in weight (Fig. 1), and had no evidence of any abnormality or deformities, and the two groups were generally indistinguishable from one another.

The animals in Group III (those that received mercaptoethylamine orally in their diet) were apathetic and failed to gain in weight (Fig. 1). They progressively developed severe deformities of the skeletal system (Fig. 2). These consisted of scoliosis of the dorsalumbar spine, bowing deformities of the tibia, and displacements through the epiphysis most marked at the upper end of the tibia and at the upper end of the humerus. In the case of the upper end of the tibia the displacement was either anterior or posterior (Fig. 3). The upper epiphysis of the humerus was always displaced inferiorly (Fig. 4) and appeared similar to "slipped femoral epiphysis" as observed in the human. There was periosteal new bone formation marked at the infraglenoid portion of the scapula, at the attachment of the triceps, at the deltoid insertion to the humerus, at the insertion of the brachialis to the ulna, and along the linea aspera of the femur.

Fig. 1.—Rats, 15 weeks old. On the left is a normal control rat. On the right near scale is a rat given mercaptoethylamine by injection. Note that they are of the same size. The center rat, given mercaptoethylamine in the diet, is deformed and extremely small in comparison.

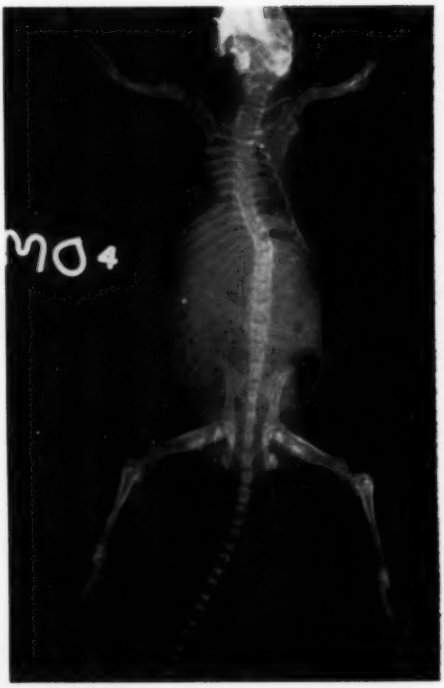
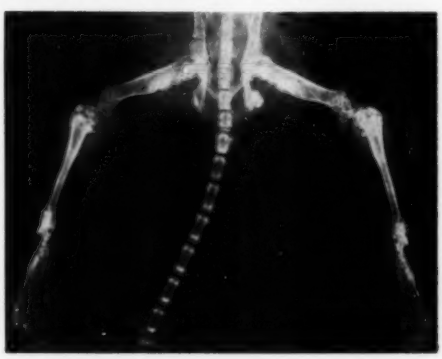


Fig. 2.—X-ray of rat after eight weeks of mercaptoethylamine in diet. Note scoliosis, irregularity and displacement of the epiphysis of both upper tibia and right humerus, and periosteal new bone at the insertion of deltoid, the infraglenoid part of left scapula, and the shafts of the femurs.

Fig. 3.—Close-up view of x-ray of the knee joints of the same rat as in Figure 2. The severe displacement and irregularity of the upper epiphysis of both tibia is well seen. Note the new bone formation in the shafts of both femurs.



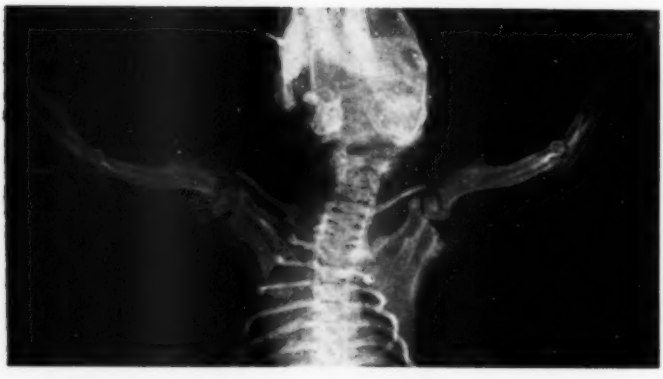


Fig. 4.—Close-up view of x-ray of the shoulder joints of the same rat as in Figure 2. The characteristic downward displacement of the right upper epiphysis of the humerus is well seen. Note the periosteal new bone at the insertion of the deltoid.

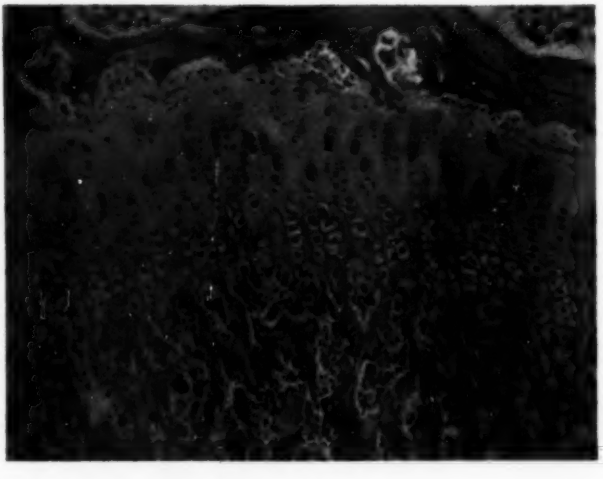


Fig. 5.—Epiphyseal plate of upper end of tibia of rat after 10 weeks of mercaptoethylamine by injection. The plate shows no abnormality. Hematoxylin and eosin; ×110.

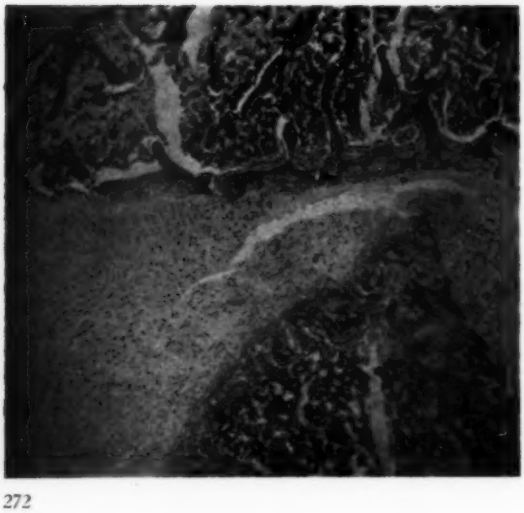


Fig. 6.—Upper end of tibia of rat after eight weeks of mercaptoethylamine in diet. Note the increased width and disorganization of the epiphyseal plate, with tear and the severe displacement and angulation between the epiphysis and the shaft. The epiphysis is at the upper part of the picture. Compare with Figure 7. Hematoxylin and eosin; ×30.

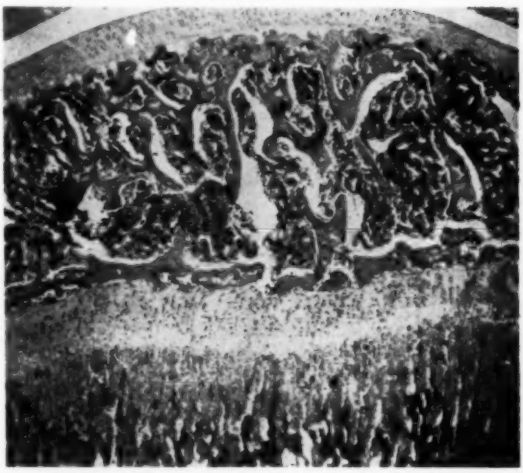


Fig. 7.—Upper end of tibia of normal rat. Part of the knee joint is seen at the upper part of the picture. The epiphysis is separated by a normal epiphyseal cartilage from the shaft and is in alignment with it. Hematoxylin and eosin; × 30.

Histologic Examination.—No skeletal lesions were noted in Group II (mercaptoethylamine by injections) when compared with the normal controls (Fig. 5). On the other hand, there were remarkable changes in Group III (mercaptoethylamine in diet), which were most marked in the epiphyseal cartilage of the upper tibia and humerus. These changes were characterized by a widening and disorganization of the epiphyseal cartilage. Rents and tears developed in the epiphyseal plate, with displacement and angulation between the shaft and the epiphysis (Figs. 6 and 7). The characteristic lesion was a great increase in the *zone of proliferating cartilage* cells, with loss of the regular columnar arrangement (Fig. 8). In addition there was some irregularity of calcification.

Histochemical studies showed linear areas in the matrix, which stained strongly with the periodic acid-Schiff reagent (Fig. 9), but no significant changes in metachromasia were noted with toluidine blue. These alterations were not seen in either the controls or Group II.

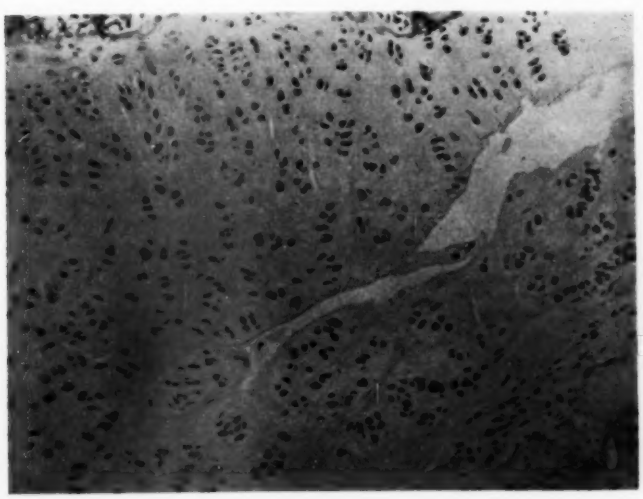


Fig. 8.—High power of same slide as in Figure 6. There is widening and disorganization of the epiphyseal cartilage, mainly due to increased width of the *zone of proliferating cartilage cells* which occupy almost the whole width of the plate. Note the tear through the plate. Hematoxylin and eosin; × 110.

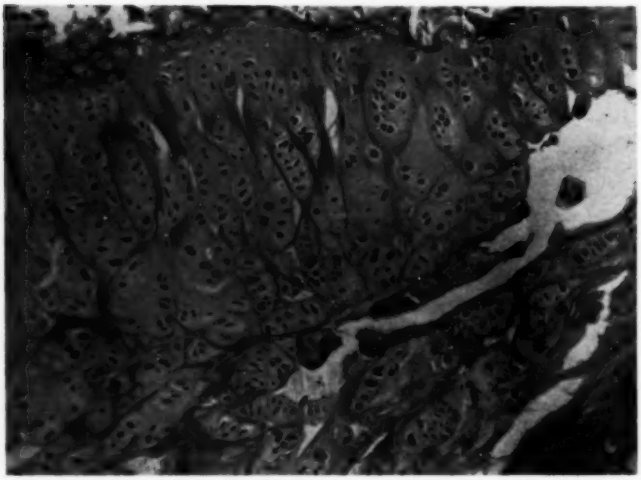


Fig. 9.—Epiphyseal plate of upper tibia after eight weeks of mercaptoethylamine in diet. Note bands of periodic acid-Schiff-positive material in the ground substance. Periodic acid-Schiff stain; ×110.

The displacement and angulation between the shaft and the epiphysis was well marked by the eighth week. Significant new bone formation was present at the periphery of the epiphyseal plate, which had become exposed due to the displacement. A free mingling of the epiphyseal cartilage cells and islands of periosteal new bone occurred at these sites. Periosteal new bone was also noted at the infraglenoid part of the scapula, at the insertion of the deltoid in the humerus, and along the linea aspera of the femur.

Comment

When rats are fed continuously a diet containing 0.5% mercaptoethylamine they develop skeletal lesions consisting of scoliosis, displacement through the epiphysial cartilage, bowing deformities of the long bones, and excess periosteal bone formation at the attachment of tendons and ligaments, which superficially resemble the lesions produced by β-aminopropionitrile (toxic Lathyrus factor).

It is remarkable that, while the continuous oral administration of mercaptoethyl-

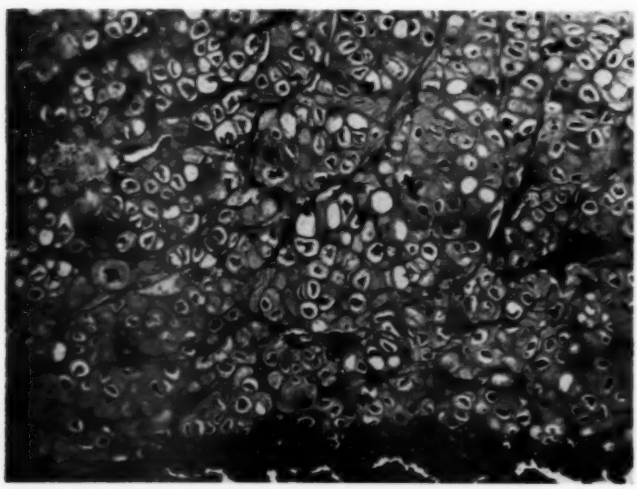


Fig. 10.—Upper end of the tibia of rat receiving dietary β-aminopropionitrile for four weeks. There is gross disorganization of the epiphyseal plate and marked widening of the *zone of maturing cartilage*. Compare with Figure 8. Hematoxylin and phloxine; ×110.

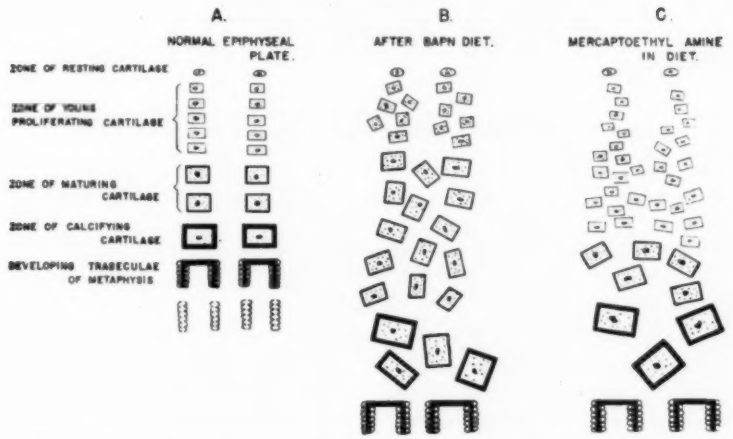


Fig. 11.—Diagramatic representation of the epiphyseal plate. *A*, normal. Note the various zones. *B*, after β-aminopropionitrile there is widening of the *zone of maturing cartilage cells*. *C*, after mercaptoethylamine there is widening of the *zone of proliferating cartilage cells*.

amine results in these skeletal lesions, no deformities or abnormalities occurred with intraperitoneal injections of even large doses of the drug.

A histochemical study[3] of the skeletal lesions produced by β-aminopropionitrile showed the disorganization of the epiphyseal plate to be due largely to a widening of the zone of *maturing cartilage cells*, with some alterations of the ground substance (Fig. 10). It was suggested that this may have been due to a block in their further differentiation and consequent interference with endochondral ossification.

On the other hand, the lesions produced by mercaptoethylamine were characterized by a widening and disorganization of the zone of *proliferating cartilage cells*, with some alteration of the ground substance, as indicated by the occurrence of linear bands of strongly positive periodic acid-Schiff material. This suggests that mercaptoethylamine interferes with further differentiation of the *zone of proliferating cartilage cells* (Fig. 11).

There are other differences in the lesions due to mercaptoethylamine. Periosteal new bone formation is not so marked, nor is scoliosis a pronounced feature in these animals. Mercaptoethylamine severely inhibits growth but is relatively less toxic than β-aminopropionitrile. Last, but not least, while mercaptoethylamine produces no lesions when given intraperitoneally and severe skeletal lesions when administered orally, β-aminopropionitrile produces identical lesions when given orally or parenterally.[4]

It is indeed a remarkable feature that a diet containing β-mercaptoethylamine produces severe skeletal lesions, while it was innocuous when given daily by intraperitoneal injection. Beta-mercaptoethylamine is an interesting compound, and its biological effects have been studied extensively.[1,5,6]

This is best summarized by Long,[7] who studied the effect of mercaptoethylamine on the tuberculin reaction in guinea pigs. He came to the conclusion that β-mercaptoethylamine had two biological actions: First, it had an *immediate action*, in which it behaved like a sulfhydryl compound. This resulted in a good reaction to tuberculin, and this effect is in keeping with its protective power in mice when given injections before irradiation. This brief action is followed by a more *prolonged action*, which is just the opposite and results in diminished sensitivity to tuberculin.

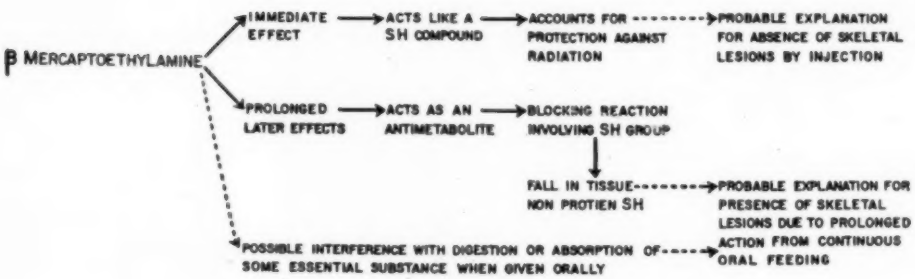


Figure 12

It was suggested that the latter effect may be due to mercaptoethylamine or one of its break-down products behaving like an antimetabolite and thus interfering with ascorbic acid or coenzyme A metabolism in some indirect manner. Though these explanations are speculative, they indicate that β-mercaptoethylamine has two biological actions, one diametrically opposed to the other.

These suggestions about β-mercaptoethylamine may be applied to the present experiment. It is quite possible that when mercaptoethylamine was injected just once a day it was rapidly metabolized [1] and its biological action was due to the "immediate effect," as postulated by Long, when it acts as a sulfhydryl compound. This might account for the absence of skeletal lesions in this group of animals.

On the other hand, when it was fed orally the organism was subjected to a prolonged effect due to the continuous feeding. Its biological action could possibly be attributed to the "prolonged later effect," postulated by Long, when it acts as an antimetabolite, and may block the essential sulfhydryl groups in the organism. This may explain the presence of skeletal lesions on oral administration. Of course it is also possible that when fed the drug may be chemically changed during digestion into a toxic compound or it may indirectly interfere with digestion or absorption of some essential substance the lack of which may cause the lesions.

These postulates are summarized in Figure 12, but further experimental data must be obtained before one of these theories may be accepted. However, the experiments reported above show that continuous oral administration of β-mercaptoethylamine results in remarkable skeletal lesions in young Wistar rats, while intraperitoneal injection of the drug once a day even in large doses does not visibly affect the animal.

Summary

Freshly weaned Wistar rats were divided into three groups. One group served as controls. The second group received 20 mg. of mercaptoethylamine intraperitoneally by injection, and the third group was maintained with a diet containing 0.5% mercaptoethylamine up to 12 weeks.

The animals that received mercaptoethylamine by injection showed no apparent abnormality as judged by radiologic, pathologic, and histologic examinations. On the other hand, the group receiving mercaptoethylamine in the diet developed severe skeletal lesions consisting of scoliosis, dislocation, and displacement through the epiphyseal plate and bowing deformity of long bones.

Histologically there was a widening and disorganization of the epiphyseal plate, which was mainly due to an increase in width of the *zone of proliferating cartilage*. There were linear areas in the ground substance of the epiphyseal cartilage which were strongly periodic acid-Schiff-positive.

The possible explanations for the different biological action of mercaptoethylamine in the two groups are discussed.

The lesions caused by mercaptoethylamine are compared with those caused by β-aminopropionitrile.

Mrs. K. Morris, Mrs. M. Hendry, Mrs. H. Cheney, and Miss K. Hoskins assisted in this study.

Department of Pathology, University of British Columbia Faculty of Medicine (8) (Dr. Taylor).

REFERENCES

1. Bacq, Z. M.; Dechamps, G.; Fischer, P.; Herve, A.; LeBihan, H.; Lecomte, J.; Pirotte, M., and Rayet, P.: Protection Against X-Rays and Therapy of Radiation Sickness with β-Mercaptoethylamine, Science 117:633 (June 5) 1953.

2. Dasler, W.: Production of Experimental Lathyrism in the Rat by 2 Different Beta-Substituted Ethylamines, Proc. Soc. Exper. Biol. & Med. 88:196 (Feb.) 1955.

3. Ramamurti, P., and Taylor, H. E.: Histolochemical Study of the Evolution and Regression of Skeletal Deformities Due to Beta-Aminopropionitril (BAPN), Lab. Invest. 7:115 (March-April) 1958.

4. Ponseti, I. V.; Wawzonek, S.; Shepart, R. S.; Ewans, T. C., and Stearns, G.: Further Studies on Lathyrism in the Rat, Proc. Soc. Exper. Biol. & Med. 92:366 (June) 1956.

5. Littman, F. E.; Carr, E. M., and Clauss, J. K.: Protection of Sulfhydryl Groups Against Ionizing Radiation, Science 125:737 (April 19) 1957.

6. Baldini, G., and Ferri, L.: Experimental and Clinical Research on the Radio-Protective Action of Cysteamine and Cystamin: II. Experimental Research upon Mammals, Brit. J. Radiol. 30:95 (Feb.) 1957.

7. Long, D. A.: The Pathogenesis of Rheumatic Fever (Milroy Lecture, Abridged), Lancet 1:529 (March 13) 1954.

Late Effects of Hypervitaminosis A in the Rat

Disturbance and Retardation in the Normal Growth of Offspring

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The acute phase of excessive administration of vitamin A to experimental animals is well known (Takahashi et al.,[1] Wolbach and Bessey,[2] Rodahl,[3] and others).

Although Cohlan [4] reported congenital abnormalities in the offspring of rats treated with excessive doses of vitamin A and other authors [5,6] described bone resorption as a result of direct effect of vitamin A on bones in tissue culture, no mention was made of the protracted effects of vitamin A administration.

In spite of high mortality, severe congenital anomalies, and poor condition of premature rats of hypervitaminotic A mothers, some offspring survived.

It is the purpose of this paper to set forth the data related to these survivals after excessive doses of vitamin A administration to pregnant rats.

Materials and Methods

In order to determine the effects of heavy doses of vitamin A on pregnant rats, groups of rats were fed orally with a solution of crystalline vitamin A alcohol in corn oil, having a potency of 500,000 I. U. per gram, at a level of 100-300 I. U. per gram of body weight per day in addition to the standard laboratory diet. For this purpose three experiments were conducted: The rats were fed with excessive doses of vitamin A before, after, and during pregnancy, respectively. Twenty young healthy pregnant rats—15 for the experimental groups and 5 as controls—were used in these experiments. Offspring of these 20 pregnant rats, 123 newborn rats in all, averaging 30 in each group, were studied for a period of four months. Records of mortality and survivors were taken in both control and vitamin-treated animals during the experiments, as indicated in Table 1.

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4th U. S. Army Medical Laboratory, Fort Sam Houston, Texas.

Results

In the first experiment, the rats of this group were fed vitamin A before pregnancy. Offspring of this group were healthy, and the vitamin A seemed to have no effects on them over a period of four months' observation.

In the second experiment, the rats were fed vitamin A after pregnancy and during the lactation. No significant difference was detectable in the rats of five litters followed about four months.

On examination, the rats of the third experiment, which received excessive doses

TABLE 1.—*Comparison of the Number of Animals and Litters in Each Group and Mortality During the Experiments*

	Number	Number Litter	Average No. Each Litter	Total No. Offspring at Birth	Offspring at End of Experiment No.	Mortality No.	Mortality %
Hypervitaminosis A *							
Group 1	5	5	6	30	24	6	20
Group 2	5	5	6½	33	28	5	15
Group 3	5	5	5	25	10	15	60
Controls	5	5	7	35	30	5	14

* Group 1 indicates animal fed with excessive doses of vitamin A before pregnancy; Group 2, after pregnancy; Group 3, during pregnancy.

TABLE 2.—*Comparison of Average Body Weight Between the Control and the Hypervitaminotic A Animals from Birth to Four Months* *

	Birth	1 Wk.	2 Wk.	3 Wk.	4 Wk.	2 Mo.	3 Mo.	4 Mo.
Hypervitaminosis A †–								
Group 1	6	14	20	35	60	150	175	215
Group 2	6½	15	20	35	65	150	185	220
Group 3	5	12	16	35	35	100	110	150
Controls	6½	15	21	35	60	150	180	225

* Body weight in grams.

† Group 1 indicates animal fed with excessive doses of vitamin A before pregnancy; Group 2, after pregnancy; Group 3, during pregnancy.

of vitamin A during the entire period of pregnancy, had premature and reduced number of litters (Table 1). Most of the offspring showed more or less severe congenital anomalies and died in a few days. The remainder, kept in observation for a period of four months, showed signs of retardation in growth and were markedly underweight (Table 2). Signs of retardation consisted of prolonged time of weaning subsequent to loss of appetite, possible retardation of development of the labial muscles, weight loss, dry skin, fragility and/or roughening of the hair, and diminished amount of subcutaneous adipose tissue. After weaning, these animals were kept in a separate cage under normal conditions and were fed the regular laboratory diet. Compared to the control rats of the same age and under the same conditions, they showed a retardation in their normal growth. At 4 months of age these rats appeared to be about one-half the size of the controls and were somewhat weaker (Fig. 1). Tables 1 and 2 show the number of animals in each litter, comparative mortality in each group, and the body weight during the experiment.

Fig. 1.—This figure shows two rats of the same age (4 months); one is normal (right), and the other (left), a subnormal offspring whose mother was fed excessive doses of vitamin A during pregnancy.

Histopathologic changes observed in hypervitaminosis A animals consisted of generalized but moderate lacunar fibro-osteoclasia of the entire bony system (Figs. 2 and 3), mild parathyroid hyperplasia, and deposits of calcium in the organs, especially in the kidneys. Detailed description of these changes will be reported elsewhere.[15]

Comment

According to the previous workers,[5,6] it is already established that vitamin A has the remarkable property of controlling the shape and texture of certain bones in young animals and has the function of controlling the position and intensity of activity of osteoclasts and osteoblasts.

Following the findings of Cohlan [4] (congenital anomalies), Giroud and Martinet [7] described considerable retardation of the growth and development of the cartilage bones in the fetuses of pregnant rats with hypervitaminosis A.

Herbertson [8] studied in tissue culture the direct action of vitamin A in chick bone and showed its reversibility in vitro. Such reversibility in vitro does not occur after

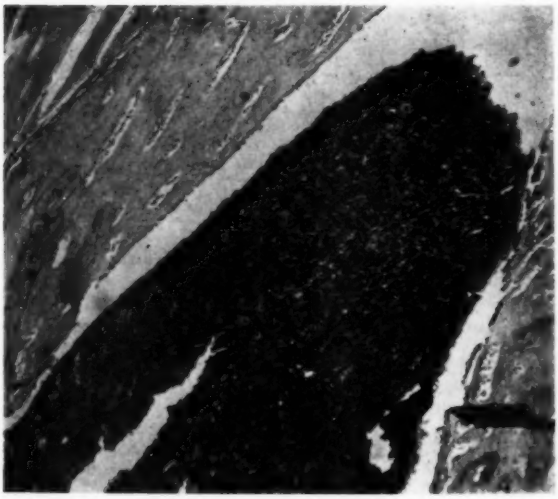

Fig. 2.—Photomicrograph showing section of normal femur in a control rat. Hematoxylin and eosin; × 60.

four months in our animals. Further study is required to determine whether or not this reversibility exists in vivo with a considerable retardation or whether vitamin A changes definitely the size of survival offspring.

Although Wolbach and his associates [9-11] in their studies of hypophysectomized, adrenalectomized, and parathyroidectomized hypervitaminotic A animals stated that the endocrine glands have no part in mediating the effects of excessive doses of vitamin A on the skeleton, other authors found evidence of changes in the endocrine glands.[12-15]

Nevertheless, the mechanism of the action of excessive doses of vitamin A in experimental animals could not be established with certainty. However, two factors seem to play a major role in hypervitaminosis A syndrome: (*a*) endocrine factor and (*b*) direct action of vitamin A on bones

Fig. 3.—Photomicrograph showing subperiosteal and subcortical lacunar fibro-osteoclasia in the femur of a hypervitaminotic A rat. The arrows show the areas of fibro-osteoclasia. Hematoxylin and eosin; × 80.

(Fell and Mellanby,[5,6] Berdjis and Rinehart [15]).

The role of the endocrine glands certainly cannot be neglected because, as mentioned above, some authors found changes in the endocrine system. From analysis of the literature relative to action of vitamin A on bone in vivo and in vitro including tissue culture,[5,6,8,15] it is believed that vitamin A has a direct action on bone. Whether or not vitamin A acts directly or by intervention of the endocrine system to change the normal growth in the offspring of animals fed during pregnancy with excessive vitamin A, heavy doses of vitamin A create a complex syndrome in the body in which the first target appears to be the bony system.

Summary

Groups of rats were fed orally with excessive doses of vitamin A before, after, and during pregnancy. Compared with the control rats of the same age, no significant difference was detectable between the offspring of the first and second groups after four months.

The rats of the third group were premature, some died and/or showed congenital anomalies, and 40% survived. The survivors (Table 1), kept under observation for a period of four months, showed signs of weakness and retardation in growth (Fig. 1). They were all underweight (Table 2).

Conclusion

It is concluded that excessive intake of vitamin A during pregnancy decreases size of offspring in rats and causes disturbance of normal growth. In addition, congenital anomalies and prematurity were observed, as described by previous authors.[4,7]

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REFERENCES

1. Takahashi, K.; Nakamiya, Z.; Kawakimi, K., and Kitazato, T.: On the Physical and Chemical Properties of Biosterin and on Its Physiological Significance, Scient. Papers Inst. Phys. & Chem. Res., Tokyo 3:81, 1925.

2. Wolbach, S. B., and Bessey, O. A.: Tissue Changes in Vitamin Deficiencies, Physiol. Rev. 22:233, 1942.

3. Rodahl, K.: Hypervitaminosis A: A Study of the Effects of Vitamin A in Experimental Animals, Skrifter No. 95, Norsk Polarinstitutt, 1950.

4. Cohlan, S. Q.: Excessive Intake of Vitamin A as a Cause of Congenital Anomalies in the Rat, Science 117:535, 1953.

5. Fell, H. B., and Mellanby, E.: The Effect of Hypervitaminosis A on Embryonic Limb-Bones Cultivated in Vitro, J. Physiol. 116:320, 1952.

6. Fell, H. B.: Effects of Excess Vitamin A on Organized Tissues Cultivated in Vitro, Brit. M. Bull. 12:35, 1956.

7. Giroud, A., and Martinet, M.: Hypervitaminosis A and Abnormalities in Rat Fetuses, Internat. Ztschr. Vitaminforsch. Bern, 28:10, 1956.

8. Herbertson, 1955, cited by Fell.[6]

9. Wolbach, S. B.: Vitamin-A Deficiency and Excess in Relation to Skeletal Growth, J. Bone & Joint Surg. 29:171, 1947.

10. Wolbach, S. B., and Maddock, C. L.: Vitamin-A Acceleration of Bone Growth Sequences in Hypophysectomized Rats, A. M. A. Arch. Path. 53:273, 1952.

11. Wolbach, S. B.; Maddock, C. L., and Cohen, J.: The Hypervitaminosis-A Syndrome in Adrenalectomized Rats: Skeletal Changes in Weanling Rats With and Without Cortisone, A. M. A. Arch. Path. 60:130, 1955.

12. Cornil, L.; Chevallier, A.; Paillas, J. E., and Chouquet, T.: Modifications of the Pituitary Body in Hypervitaminosis and Hypovitaminosis A, Ann. Anat. path. 16:83, 1939.

13. Sadhu, D. P., and Brody, S.: Excess Vitamin A Ingestion, Thyroid Size and Energy Metabolism, Am. J. Physiol. 149:400, 1947.

14. Uotila, U.: Über die Schilddrüsenveränderungen bie A-, B-, C- und D-Avitaminosen, Arch. path. Anat. 301:535, 1938.

15. Berdjis, C. C., and Rinehart, J. F.: Fibro-Osteoclasia in the Rat and Guinea Pig Following Excessive Administration of Vitamin A, Acta vitaminol. 12:49, 1958.

Pathologic Findings in a Case of Panhypopituitarism and Diabetes Insipidus

Comments Relative to Hypothalamic, Pituitary, and "End-Organ" Interrelationships

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It is well recognized that destructive lesions of the hypophysis, such as infarct necrosis, metastatic carcinoma, and a variety of inflammatory processes, account for the vast majority of instances of panhypopituitarism. Indeed, Sheehan and Summers,[1] in their inclusive review of the pathologic findings in examples recorded up to 1949, failed to indicate any case in which the pituitary might be considered morphologically unaltered, although they made reference to several in which the changes appeared "trivial" or of doubtful significance. It is to be noted that histologic examination of these latter was limited to routine oversight staining techniques, and there is no information available concerning the quantitative relationships of the various hypophyseal cells in this endocrine state in man. There is also a paucity of histologic information relative to the hypothalamus in panhypopituitarism, although the significance of relationships between this vital center and the adenohypophysis has been demonstrated experimentally.[2-9]

Similarly, collective reviews dealing with the etiology of diabetes insipidus in man emphasize the frequency of destructive lesions involving the hypothalamus, pituitary stalk or posterior lobe of this gland.[10-13] Although examples have been designated as idiopathic in nature, description of their histologic features has been meager and we have encountered only one previously recorded instance of diabetes insipidus in which the neurohypophysis appeared intact.[13] This was a case in a 3½-month-old infant. Although the brain was extensively studied, no comments concerning antidiuretic substance were made.

The purpose of this report is to relate the clinical and pathologic findings in a 47-year-old white man with panhypopituitarism and diabetes insipidus. Although an unusual lesion involving portions of the posterior and ventromedial hypothalamic nuclei and adjacent fiber tracts were encountered, the neurohypophyseal system concerned with the elaboration of antidiuretic hormone and adenohypophysis was morphologically intact. Detailed pathologic study of the hypothalamus and pituitary, including a differential count of the various hypophyseal cells, has provided information concerning the interrelationship of these structures which has heretofore received only scant attention in man. In addition, information has been disclosed relative to other parameters of hypophyseal function which are worthy of note.

Report of a Case

The patient, a 47-year-old white man, was admitted to the hospital three years prior to death because of migratory pain of the hips, shoulders, ankles, knees, elbows, and wrist which was accompanied by fatigue and moderate anorexia unassociated with loss of weight. He also experienced mild polyuria, nocturia, polydipsia, and thirst. The latter was relieved by the ingestion of iced water. The patient stated the onset of these symptoms was abrupt, having occurred one year prior to admis-

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sion. He had been married twice but had no offspring.

Examination revealed his height to be 5 ft. 7 in. and weight, 135 lb. His temperature was 99.2 F; pulse, 80 per minute, and blood pressure, 80/60 mm. Hg. Although he was well developed and nourished, he appeared older than his stated age. There were two vitiliginous areas on the dorsum of the penis, and the testes were small and soft. The prostate was small and firm. A slight fusiform swelling of the proximal interphalangeal joints was also evident.

Laboratory examination revealed a hemoglobin value of 11 gm. %; WBC, 5500 per cubic millimeter, with 62% lymphocytes, 20% eosinophils, and 18% neutrophils. On several occasions up to 3% "blast" forms were noted in the peripheral blood. Bone marrow aspirates also revealed a slight increase of immature forms. Platelet counts and bleeding and coagulation studies were within normal limits. Repeated urinalyses were normal except for specific gravities, which ranged from 1.002 to 1.006. A P. S. P. test revealed 32% excretion at 15 minutes and 51% at 1 hour. Fasting blood sugar was only 54 mg. %, and an oral glucose tolerance test revealed 105 mg. % at one-half hour, 105 mg. % at one hour, 110 mg. % at two hours, and 80 mg. % at three hours. Other blood chemistry determinations, liver-function tests, serologic tests for syphilis, blood cultures, and stool examinations were either within normal limits or negative. A B. M. R. was −31%, and P. B. I. was 3.2μg. %. Urinary 17-ketosteroids for 24 hours was 3.4 mg. (low). No urinary gonadotropins could be detected by bioassay. Roentgenograms of the skull, chest, and peripheral joints were normal, as was the ECG.

The patient received a sodium-restricted diet and cortisone, 75 mg. per day. This resulted in a remission from joint pain and a decrease of eosinophilia to normal levels, a gain in body weight, and improvement in his general condition. The specific gravity of the urine increased to 1.010 after an injection of 1 ml. of vasopressin (Pitressin) tannate in oil, and the urinary output remained less than 2 liters per day. There was no change observed in the peripheral lymphocytosis. He was treated as an outpatient for the next four months.

Discontinuance of cortisone was promptly followed by fever and arthritic manifestations. Repository corticotropin injection (ACTH Gel), 20 units per day, was administered and was followed by a remission from these symptoms. He also received desiccated thyroid, 60 mg. per day; methyltestosterone, 10 mg. per day, and vasopressin tannate, once weekly. He did well with this therapeutic regimen for approximately two and one-half years and was able to resume work as a clerk. However, he again experienced fever, polyuria, polydipsia, and malaise and was readmitted to the hospital.

Examination at this time revealed his skin to be smooth and dry, with a few petechiae present in the skin of the chest. There was no axillary hair, and pubic hair was scant. His blood cell count was similar to that noted previously, including the persistent lymphocytosis with a few "blast" forms being present. Blood chemical studies and urinalyses were also similar to those recorded previously. His B. M. R. was now +5. He continued to receive cortisone, vasopressin, methyltestosterone, and desiccated thyroid; 6-chloropurine (250 mg. t. i. d.) was also administered but was discontinued after one week because of nausea and vomiting. Despite therapy the patient exhibited an ingravescent course characterized by extreme weakness, loss of weight, nausea, vomiting, and purpura over the extremities, and he died in coma three years after his first hospitalization.

Necropsy Examination

Macroscopic Findings

The body appeared well developed. Petechiae were present in the skin of the arms, chest, and abdomen. Axillary hair was absent, and a feminine escutcheon was evident.

The lungs were edematous, weighing 900 gm.

Petechiae were present in the pericardium and serosa of the gastrointestinal tract. Ulceration of the mucosal surface of the distal third of the esophagus was evident.

There was minimal atherosclerosis of the aorta and coronary arteries.

The liver weighed 1750 gm. Its cut surface was uniform, revealing centrolobular congestion.

The prostate was small and firm, weighing only 12 gm. The testes together weighed 18 gm. Their tubules could not be plucked.

The combined weight of the adrenals was only 6 gm. Their cut surfaces revealed thin brown cortices. The medullary zones were not remarkable.

The thyroid weighed 12 gm. Irregular bands of dense white tissue coursed throughout the parenchyma.

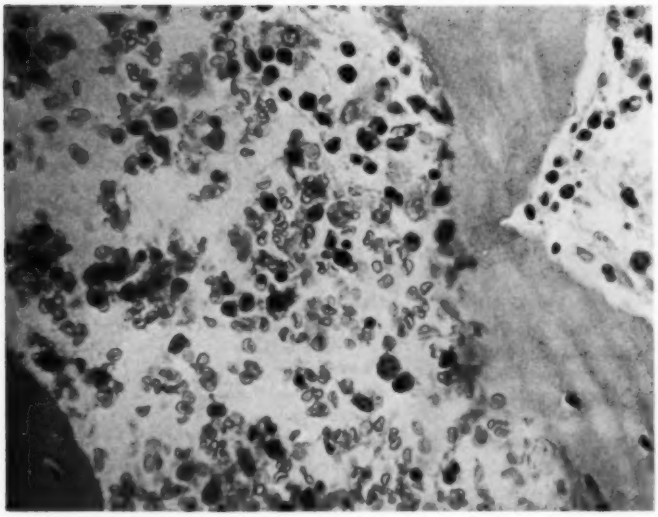


Fig. 1.—Section of vertebral bone marrow revealing paucity of cellular elements. Plasma cells, nucleated red cells, few immature granulocytic elements, and rare reticulum cells are evident. Reduced 20% from mag. × 450.

Three parathyroids were recovered from the peritracheal tissue. Their combined weight was 40 mg.

The pituitary gland weighed only 300 mg. but was of normal configuration. No abnormality of the sella turcica was apparent. The pituitary stalk was thin, but intact.

The brain weighed 1300 gm. Its external and cut surfaces were not remarkable. The hypothalamus was coronally sectioned at 3 mm. intervals. No macroscopic lesion was evident.

Microscopic Findings

Sections of heart revealed focal areas of myocardial fibrosis. A rare arteriole exhibited focal degeneration of the wall, with a subendothelial hyaline mass protruding into the lumen.

Sections of bone marrow obtained from rib, sternum, and vertebrae were all hypoplastic (Fig. 1). Small foci of normo-

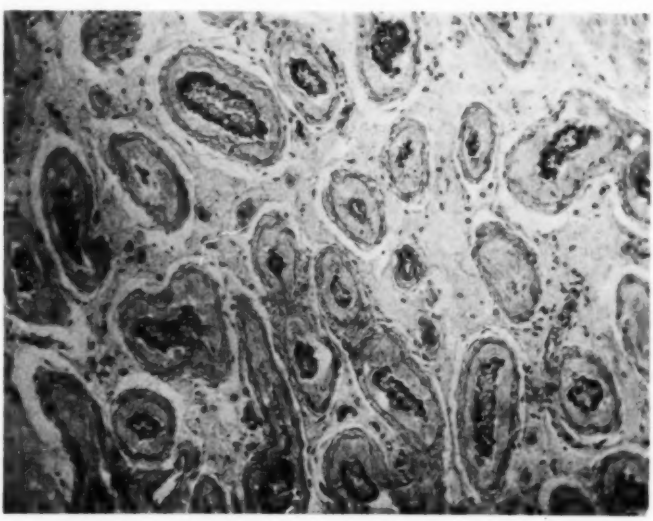


Fig. 2.—Marked hyalinization of tubules of testis. Only Sertoli cells are evident. Reduced 20% from mag. × 90.

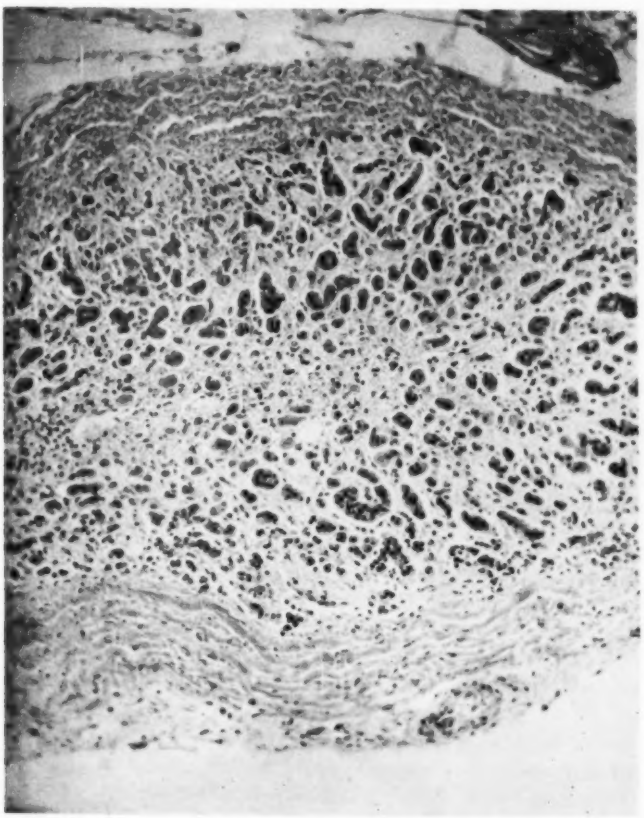


Fig. 3.—Adrenal cortex comprised of short cords of nonvacuolated cells; × 95.

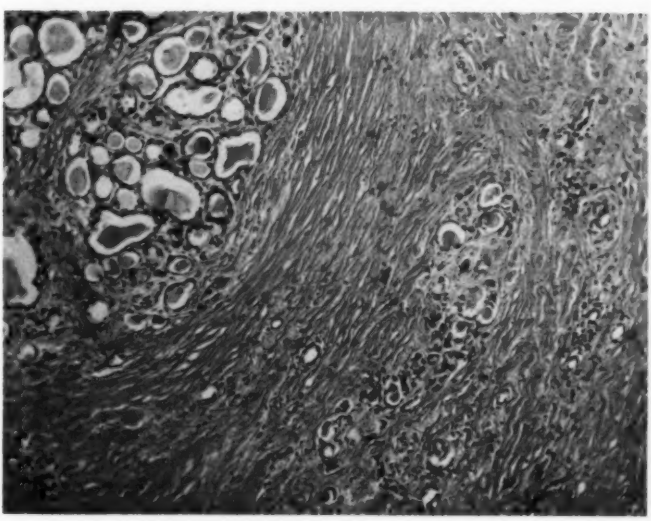


Fig. 4.—Broad bands of connective tissue separate thyroid lobules and their remnants. Acini are lined by flattened epithelium. Reduced 20% from mag. × 75.

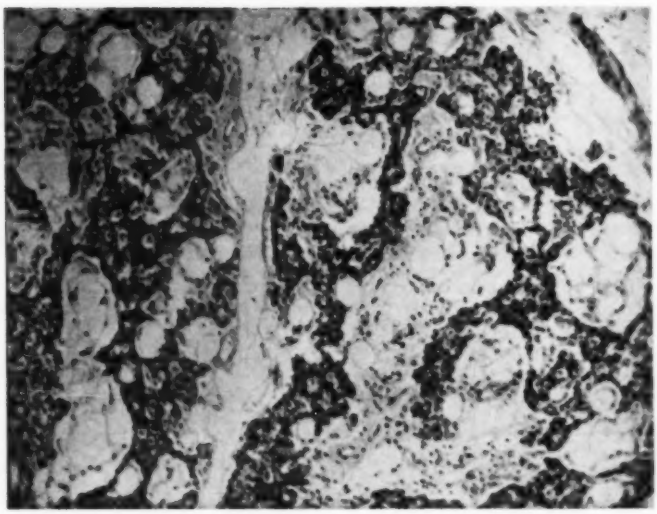


Fig. 5.—Narrow cords of parathyroid cells separated by adipose tissue. Reduced 20% from mag. ×75.

blasts, reticulum cells, granulocytic elements, and megakaryocytes were noted, being separated by adipose tissue. Plasma cells were also evident.

Sections of spleen revealed rare immature granulocytic elements scattered throughout the red pulp.

Ulceration of the esophageal mucosa was evident. Candida and bacterial masses associated with a slight neutrophil infiltrate were present.

Prostatic glands appeared simplified, being lined by a solitary layer of flattened cuboidal epithelium.

Sections of testes revealed marked hyalinization of the tubules (Fig. 2). In most instances only a few Sertoli cells could be identified lining the latter. Hyalinization of

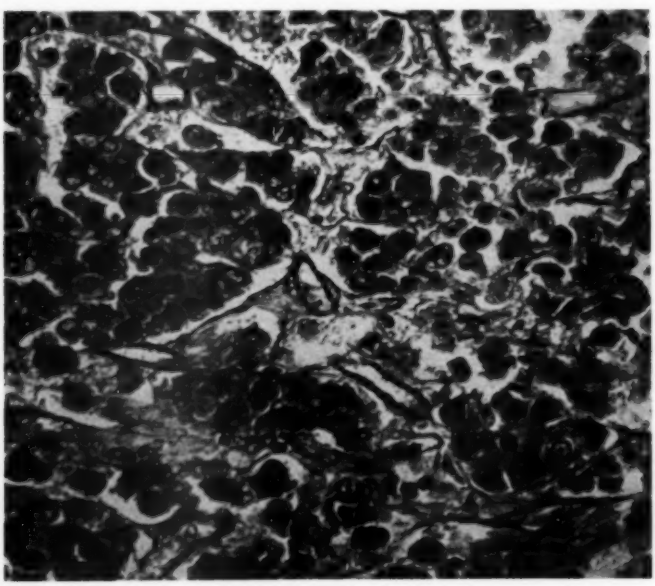


Fig. 6.—Section of adenohypophysis revealing basophil hyperplasia (appear dark) and scattered amphophils (indistinct cytoplasm.). Reduced 10% from mag. ×425.

Differential Count of Cells of Anterior Pituitary *

	Case, %	Normal, %
Chromophobes	10.7	50
Acidophils	52.1	30–40
Basophils	22.9	5–10
Amphophils	13.4	0.5
Hypertrophic amphophils	1.3	1

* Based on count of 8,000 cells.

the interstitial tissue was also evident, and Leydig cells were extremely sparse. No spermatozoa could be found.

The adrenal cortices were comprised of short cords of cells with granular cytoplasm (Fig. 3). Zonation was not apparent. The medullary portions appeared normal.

Sections of thyroid revealed large irregular zones of fibrosis (Fig. 4). Remaining follicles were lined by flattened cuboidal epithelium and contained dense colloid. In some areas aggregates of lymphocytes were noted.

The parathyroid glands were comprised of narrow cords of principal cells, three or four layers in thickness, separated by abundant adipose tissue (Fig. 5).

The pituitary gland appeared morphologically intact. However, a diminution in cell

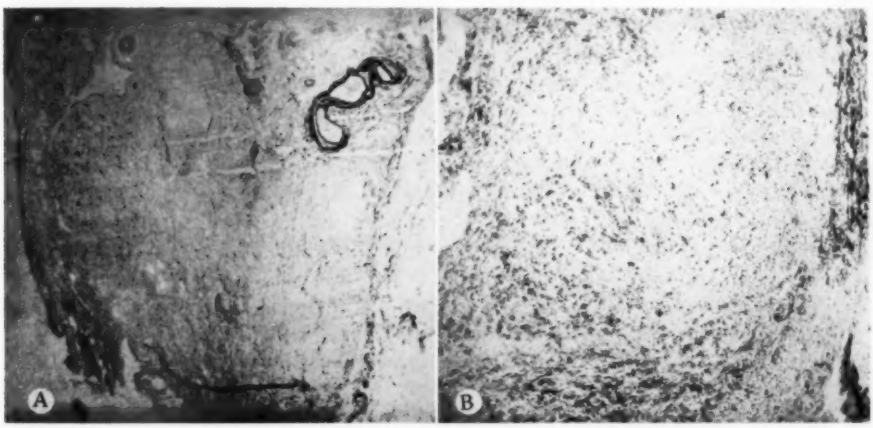
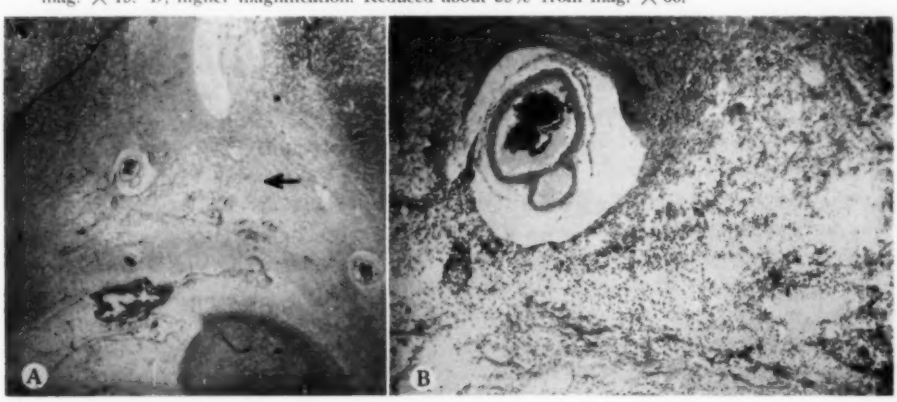


Fig. 7.—*A*, hypophyseal stalk from patient with diabetes insipidus and panhypopituitarism stained by aldehyde fuchsin technique. There is no stainable material evident as compared to control, *B*. Reduced about 35% from mag. × 75.

Fig. 8.—*A*, coronal section of hypothalamus at posterior level of ventromedial nuclei stained by Lillie-Weil-Weigert method, revealing focus of encephalomalacia. Reduced about 35% from mag. × 15. *B*, higher magnification. Reduced about 35% from mag. × 60.



population of the adenohypophysis was evident. Differential cell count of 8000 adenohypophyseal cells in sections stained by the periodic acid-Schiff (PAS)-orange G technique [14] revealed a marked amphophil and basophil hyperplasia (Fig. 6). Chromophobes were decreased and acidophils only moderately increased (Table).

The posterior lobe appeared smaller than usual, and the pituitary stalk, somewhat narrower. Sections which were stained by the aldehyde fuchsin and chrome alum hematoxylin techniques [15,16] revealed an absence of fuchsinophilic or hematoxylinophilic material in the stalk (Fig. 7) and posterior pituitary, whereas this material was abundant in control sections.

Examination of the hypothalamus by step sections of serial blocks revealed a discrete focus of encephalomalacia involving the mamillothalamic tract, supramammary decussation, part of the fornix, and inferior aspects of the posterior and ventromedial hypothalamic nuclei (Fig. 8). The lesion was accompanied by only minimal gliosis, and surrounding tissue revealed recent hemorrhage. Vascular alterations were not apparent. Myelin sheaths, as revealed by the Lillie-Weil-Weigert stain, or Nissl substance in sections stained with thionin appeared unaltered in the neurons of the paraventricular or supraoptic nuclei or their associated fiber tracts. Although fuchsinophilic and hematoxylin granules were present in ganglion cells of the paraventricular and supraoptic nuclei, the significance of this finding could not be evaluated, since similar granules could be identified in practically all neurons in cerebral and spinal cord nuclei which contained lipofuscin pigment. Discrete granules could not be discerned along the fiber tracts emanating from these anterior hypothalamic nuclei. However, again this finding could not be evaluated, since sections from several control hypothalami which were similarly processed and stained also failed to reveal any neurosecretory substance in such locations.

Comment

The appearance of the parathyroids, thyroid, adrenal cortices, and testes in this patient with panhypopituitarism is similar to the descriptions of these organs in previously recorded examples of this endocrine disorder. The absence of urinary gonadotropins and the favorable therapeutic response to corticotropin exhibited by this patient represent evidence that the changes observed in these target organs were the result of adenohypophyseal failure rather than a primary pluriglandular insufficiency.[17,18] Although there is no conclusive evidence for the presence of a parathyrotropic hormone, the atrophic change observed in the parathyroids in this and other examples of panhypopituitarism [1] is strongly suggestive that the hypophysis exerts a significant influence on their function and structure. Some experimental evidence has been provided which would indicate that hypophyseal secretion results in an elevation of serum phosphorus and as a consequence induces parathyroid activity.[19] Atrophy of the parathyroid glands has been attributed to the deprivation of this pituitary function. The marked reduction of adrenal cortical function associated with panhypopituitarism appears sufficient to protect persons with this state from developing hypocalcemic tetany by eliminating the inhibitory effect of corticoids on calcium absorption or its augmentation of calcium excretion.[20,21] The cords of adrenal cortical cells which were present would appear to be related, at least in part, to aldosterone secretion, since the elaboration of this adrenal cortical hormone is not considered to be predominantly under hypophyseal control.[22] This consideration might well explain the lack of significant electrolyte aberrations in this and other instances of panhypopituitarism.

Although the hypothalamus probably does not exclusively regulate adenohypophyseal function, its significance in this respect has been indicated by various experimental studies.[2-9] Since there are relatively few

fiber tracts which may be traced from this vital center into the adenohypophysis, it is considered most likely that this effect is mediated through the elaboration of neurosecretory substance which gains entrance into the hypophyseal portal system.[23,24] The destruction of portions of the posterior and ventromedial hypothalamic nuclei and adjacent fiber tracts observed in the patient presented correlates with those experimental studies relating various hypothalamic sites with adenohypophyseal function.[2-9] The atrophy of the hypophysis and failure to find any destructive lesion in the adenohypophysis of this patient emphasizes the significant role of the hypothalamus in adenohypophyseal function. Since the median eminence was intact, it becomes apparent that involvement of this structure is not essential for the production of panhypopituitarism. Although the etiology of the hypothalamic lesion encountered is obscure, it is not unlikely that it was the result of a vascular alteration, albeit such change was not apparent in the many sections studied. We know of no discrete demyelinating disease which might account for the changes observed.

As indicated previously, information concerning quantitative relationships of the various hypophyseal cells in panhypopituitarism in man has not been previously presented. Limited studies in this regard in animals with experimental hypothalamic lesions have revealed either a normal distribution of pituitary cells [4] or a diminution in those forms considered to be responsible for the elaboration of gonadotropin.[3] The presence of amphophil and basophil hyperplasia of the pituitary in this patient at first consideration appears to represent a paradox, since the amphophil has been considered to represent an actively secreting form of all hypophyseal cells and may be responsible for the elaboration of the various tropic hormones of the adenohypophysis, although not simultaneously.[25] Indeed, amphophil hyperplasia has been described in various conditions related to hypopituitarism.[25] On the other hand, Russfield has observed a similar preponderance of this cell type in the pituitary glands of several dwarfs.[26] She concluded that in these instances the amphophil either was incapable of producing somatotropic hormone or, if present, its utilization was impaired. The failure to recover gonadotropin and the response to exogenous corticotropin would indicate that the latter circumstance was not present in this patient with panhypopituitarism and is in keeping with the well-recognized endocrine aphorism that hyperplasia and hypersecretion are not synonymous. The observation of Guillemin [27] that explants of adenohypophysis failed to produce corticotropin unless hypothalamic tissue was added suggests that hypothalamic secretion or substance is essential for the elaboration of at least some adenohypophyseal hormones. The adenohypophyseal hyperplasia might then be analogous to that observed in the thyroid subsequent to iodine deficiency. A similar interpretation may be derived from the observation of Bogdanove et al.,[3,4] who noted decreased hypophyseal gonadotropin in rats with hypothalamic lesions productive of gonadal atrophy. Although the basophil hyperplasia may in part be attribued to the cortisone received by this patient, it is of interest that Crooke's cells, not infrequently noted after such therapy,[25] were not encountered.

Although such diagnostic tests for diabetes insipidus as the administration of hypertonic saline or nicotine were not performed in this patient, the sudden onset of polyuria, desire for iced water, and favorable response to vasopressin appear to represent sufficient clinical evidence for the presence of diabetes insipidus. The relatively mild state of diabetes insipidus experienced by this patient may be accounted for by the associated insufficiency of adrenal cortical and thyroid hormones, since both are considered to possess diuretic action.[12,28-30] This consideration has been offered to explain the infrequent associa-

tion of panhypopituitarism and diabetes insipidus.

It appears significant that the paraventricular and supraoptic hypothalamic nuclei and fiber tracts were not involved by the lesion encountered in the more posterior portion of the hypothalamus in this patient; yet, there is abundant evidence which relate the former sites to the elaboration of antidiuretic hormone.[24,31,32] The identification of the latter in the paraventricular and supraoptic nuclei by both the aldehyde fuchsin and the chrome alum hemotoxylin techniques cannot be made with certainty, since it appears from the results of this limited study that similar tinctorial properties are exhibited by all ganglion cells or neurons possessing lipofuscin pigment. On the other hand, the lack of stainable material in the hypophyseal stalk and posterior pituitary observed in this patient appears to represent more valid evidence that an insufficiency of antidiuretic hormone existed. This information suggests that alterations of the nuclei and fiber tracts in one portion of the hypothalamus may significantly effect the function of those nuclei in other regions of this structure. Such a concept is in keeping with those anatomical studies which emphasize the intricate communications of hypothalamic fibers and the poor delineation of nuclear cells in the human hypothalamus.[6]

The hematological findings in this patient, although suggestive of leukemia, appear to be adequately explained on the basis of panhypopituitarism. The failure to observe organ infiltration and the presence of a hypoplastic bone marrow at necropsy substantiate this conclusion. The lack of anatomical evidence to provide a diagnosis of leukemia in this instance cannot be attributed to the antileukemic therapy utilized. Only a relatively small dose of 6-chloropurine was administered, which in our experience has been insufficient to eradicate leukemic deposits or produce a hypoplastic bone marrow. Anemia, which was exhibited by this patient, is a well-recognized accompaniment of human and experimental hypopituitarism,[33,34] being attributed to the loss of a hypophyseal erythropoietic factor[35,36] as well as end-organ insufficiency, particularly thyroidal and adrenal cortical hormones. Similarly, a slight to moderate neutropenia with eosinophilia and a relative lymphocytosis and a hypoplastic bone marrow have been frequently described in panhypopituitarism.[33] The occurrence of immature hematopoietic forms in the peripheral blood and bone marrow which this patient demonstrated has received less attention. It is pertinent, however, that Greig and associates[33] described a marked increase in "lymphoid reticulum cells" in the bone marrow preparations from a patient with panhypopituitarism. The cells encountered in the marrow preparations from the patient of this report are morphologically compatible with reticulum cells, and clusters of these cells could be identified in the marrow sections obtained at necropsy.

The occurrence of arthritic manifestations which initially prompted this patient to seek medical attention has also been noted previously in patients with panhypopituitarism.[1] The synovia of the knee joints failed to disclose any pathologic alterations at necropsy which might relate his symptoms to any particular type of arthritis. A similar experience has been noted in patients with Addison's disease with arthritic complaints.[37] Hypophyseal adrenal cortical relationships are obvious which may account for the arthritis noted in patients with panhypopituitarism. Both corticotropin and cortisone relieved the arthritic complaints of the patient in this report, and Pearse[38] has noted hypophyseal changes in patients with rheumatoid arthritis similar to those observed in persons with Addison's disease.

Summary

Necropsy examination of a 47-year-old white man with clinical manifestations of panhypopituitarism and diabetes insipidus

revealed a discrete focus of encephalomalacia involving portions of the ventromedial and posterior hypothalamic nuclei and adjacent fiber tracts. No morphologic alterations of the pituitary or its stalk were evident, although the former was moderately atrophic. The etiology of the hypothalamic lesion could not be demonstrated, although it appears most likely to be the result of vascular change. The appearance of the target endocrine organs and pertinent experimental considerations indicate the significance of this hypothalamic area in regulating adenohypophyseal function.

A differential count of the various types of hypophyseal cells revealed a marked amphophil and basophil hyperplasia. This finding is interpreted as representing a nonfunctional hyperplasia analogous to that observed in other endocrine states.

The failure to demonstrate antidiuretic substance in the hypophyseal stalk by the aldehyde fuchsin and chrome alum hematoxylin techniques represented the only positive histologic evidence of diabetes insipidus. The difficulty in evaluating the state of neurosecretory material in the supraoptic and paraventricular hypothalamic nuclei in adult humans by these tinctorial reactions is indicated and is attributed to the false-positive reactions observed in ganglion cells containing lipofuscin pigment. The absence of a lesion in the anterior hypothalamic nuclei concerned with the elaboration of antidiuretic substance suggests that functional intercommunications exist between the various hypothalamic nuclei and fiber tracts.

The hematologic abnormalities associated with panhypopituitarism which may simulate leukemia are indicated, and note of the relationship of this endocrine disorder and arthritis is made.

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REFERENCES

1. Sheehan, H. L., and Summers, V. K.: The Syndrome of Hypopituitarism, Quart. J. Med. 18:319, 1949.

2. Greer M. A., and Erwin, H. L.: Evidence of Separate Hypothalamic Centers Controlling Corticotropin and Thyrotropin Secretion by the Pituitary, Endocrinology 58:665, 1956.

3. Bogdanove, E. M., and Halmi, N. S.: Effects of Hypothalamic Lesions and Subsequent Propylthiouracil Treatment on Pituitary Structure and Function in the Rat, Endocrinology 53:274, 1953.

4. Bogdanove, E. M.; Spirtos, B. N., and Halmi, N. S.: Further Observations on Pituitary Structure and Function in Rats Bearing Hypothalamic Lesions, Endocrinology 57:302, 1955.

5. Laquer, G. L.; McCann, S. M.; Schreiner, L. H.; Rosemberg, E.; Rioch, D. McK., and Anderson, E.: Alterations of Adrenal Cortical and Ovarian Activity Following Hypothalamic Lesions: Based on Eosinophile Response, Hormone Assay and Histological Examination, Endocrinology 57:44, 1955.

6. Ingraham, W. R.: The Hypothalamus, Clin. Symposia 8:117-156, 1956.

7. Barrnett, R. J., and Greep, R. O.: The Pituitary Gonadotropic Activity of Stalk-Sectioned Male Rats, Endocrinology 49:337, 1951.

8. Ganong, W. F.; Fredrickson, D. S., and Hume, D. M.: The Effect of Hypothalamic Lesions on Thyroid Function in the Dog, Endocrinology 57:355, 1955.

9. Engel, F. L.: The Adenohypophysis, in Clinical Physiology, edited by Arthur Grollman, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1957, Chap. 22, p. 582.

10. Welt, L. G.: The Posterior Lobe of the Pituitary, in Clinical Physiology, edited by Arthur Grollman, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1957, Chap. 23, p. 595.

11. Jones, G. M.: Diabetes Insipidus: Clinical Observations in 42 Cases, Arch. Int. Med. 74:81, 1944.

12. White, A. G.: Diabetes Insipidus: A Discussion, Including a Reexamination of the Site and Mode of Action of the Antidiuretic Hormone, J. Mt. Sinai Hosp. New York 22:15, 1955.

13. Cannon, J. F.: Diabetes Insipidus: Clinical and Experimental Studies with Consideration of Genetic Relationships, A. M. A. Arch. Int. Med. 96:215, 1955.

14. Pearse, A. G. E.: Differential Stain for the Human and Animal Anterior Hypophysis, Stain Technol. 25:95, 1950.

15. Gomori, G.: Aldehyde-Fuchsin: A New Stain for Elastic Tissue, Am. J. Clin. Path. 20:665, 1950.

16. Gomori, G.: Observations with Differential Stains on Human Islets of Langerhans, Am. J. Path. 17:395, 1941.

17. Howard, R. P.; Mills, E. S., and Mathews, W. H.: Pluriglandular Insufficiency Without Panhypopituitarism, J. Clin. Endocrinol. 12:974, 1952.

18. Rupp, J. J., and Paschkis, K. E.: Pluriglandular Insufficiency Simulating Panhypopituitarism: Report of 2 Cases, Am. J. Med. 18:507, 1955.

19. Törnblom, N.: On the Functional Relationship Between the Pituitary Gland and the Parathyroids, Acta endocrinol., Supp. 4, 1949.

20. Leifer, E., and Hollander, W., Jr.: Idiopathic Hypoparathyroidism and Chronic Adrenal Insufficiency: A Case Report, J. Clin. Endocrinol. 13: 1264, 1953.

21. Perlmutter, M.; Ellison, R. R.; Norsa, L., and Kantrowitz, A. R.: Idiopathic Hypoparathyroidism and Addison's Disease, Am. J. Med. 21: 634, 1956.

22. Conn, J. W., and Fajans, S. S.: The Adrenal Cortex, in Clinical Physiology, edited by Arthur Grollman, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1957, Chap. 26, p. 648.

23. Engel, F. L.: The Adenohypophysis, in Clinical Physiology, edited by Arthur Grollman, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1957, Chap. 22, p. 562.

24. Sharrer, E., and Sharrer, B.: Hormones Produced by Neurosecretory Cells, Recent Progr. Hormone Res. 10:183, 1954.

25. Russfield, A. B.: The Adenohypophysis, in Analytical Pathology, edited by Robert C. Mellors, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1957, Chap. 5, p. 293.

26. Russfield, A. B., and Reiner, L.: The Hypophysis in Human Growth Failure: Report of 3 Dwarfs, Lab. Invest. 6:334, 1957.

27. Guillemin, R., and Rosenberg, B.: Humoral Hypothalamic Control of Anterior Pituitary: A Study with Combined Tissue Cultures, Endocrinology 57:599, 1955.

28. Gaunt, R.; Birnie, J. H., and Eversole, W. J.: Adrenal Cortex and Water Metabolism, Physiol. Rev. 29:281, 1949.

29. Blotner, H., and Cutler, E. C.: Total Thyroidectomy in the Treatment of Diabetes Insipidus, J. A. M. A. 116:2739, 1941.

30. Nagareda, C. S., and Gaunt, R.: Functional Relationship Between the Adrenal Cortex and Posterior Pituitary, Endocrinology 48:560, 1951.

31. Groethuysen, U. C.; Clark, E. C.; Randall, R. V., and Dodge, H. W., Jr.: The Neurogenic Basis of Diabetes Insipidus: A Review, Proc. Staff Meet. Mayo Clin. 32:90, 1957.

32. Billenstien, D. C., and Leveque, T. F.: The Reorganization of the Neurohypophyseal Stalk Following Hypophysectomy in the Rat, Endocrinology 56:704, 1955.

33. Greig, H. B. W.; Metz, J., and Sunn, L.: Anaemia in Hypopituitarism: Treatment with Testosterone and Cortisone, South African J. Lab. & Clin. Med. 2:52, 1956.

34. Killman, S-A.: A Case of Pancytopenia in Hypopituitarism, with a Review of the Hematological Changes in Clinical and Experimental Pituitary Insufficiency, Acta haemat. 15:34, 1956.

35. Meineke, H. A., and Crafts, R. C.: Effects of Combined Thyroxin-Cortisone-Growth Hormone Therapy on Hematopoiesis in Hypophysectomized Rats, Proc. Soc. Exper. Biol. & Med. 96:74, 1957.

36. Van Dyke, D. C.; Simpson, M. E.; Contopoulos, A. N., and Evans, H. M.: The Separate Existence of the Pituitary Erythropoietic Hormone, Blood 12:539, 1957.

37. Selye, H.: The Physiology and Pathology of Exposure to Stress: A Treatise Based on the Concepts of the General-Adaptation-Syndrome and the Diseases of Adaptation, Montreal, Acta, Inc., 1950, p. 386.

38. Pearse, A. G. E.: The Hypophysis in Rheumatoid Arthritis, Lancet 1:954, 1950.

Metastasis of Cancer to Cancer

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Recently, several reports have appeared describing the metastasis of one cancer to another. This curious situation, related to the occurrence of multiple primary malignancy, had attracted comment as early as the turn of the century. In 1902, Berent [1] reported a case of squamous-cell carcinoma of the jaw which metastasized remotely to a hypernephroma of the right kidney as well as to the regional cervical lymph nodes. A full generation elapsed before Hammann,[2] in 1927, described a case of probable carcinoma of the thyroid with widespread metastases and tumor implants in a hypernephroma. In 1928, Walter [3] observed metastatic deposits from an endometrial carcinoma in a hypernephroma of the right kidney; Schmorl [4] described secondary involvement of a hypernephroma by metastases from a carcinoma of the lung; Simard and Saucier [5] reported a rhabdomyosarcoma of the mesentery with secondary deposits from a prostatic carcinoma. It is noteworthy that the host cancer was a hypernephroma in four of the first five cases. No additional instances were recorded until Walther,[6] in 1948, wrote of three cases with a hypernephroma as the recipient malignancy. The primary carcinoma, which was widely disseminated in each instance, originated in the lung, stomach, and pyriform sinus, respectively. He had encountered three additional cases by 1954; these, as cited by Rabson and his associates,[7] were as follows: an adenocarcinoma of the thyroid, metastasizing to an adenocarcinoma of the stomach; a bronchogenic carcinoma, to an adenocarcinoma of the prostate, and a small-cell carcinoma of the bronchus, metastasizing to an adenocarcinoma of the pancreas. The primary tumor in each instance was widely disseminated. Ortega et al.[8] described involvement of a hypernephroma by a widely metastasizing melanoma of choroidal origin. His two other cases, in which leukemic nodes regional to a carcinoma harbored metastases, do not seem germane to this report, since they merely demonstrate that leukemia does not alter the pathway of lymphatic drainage. Recently, Rabson et al.[7] contributed five more cases, four with hypernephroid carcinoma as the recipient malignancy. The secondary growths originated from the lung in two cases and from the prostate in two cases. In his fifth case, metastases of a cecal adenocarcinoma lodged in lymphosarcomatous deposits in lung, lymph nodes, pancreas, and liver. Another case of adenocarcinoma of the prostate which metastasized solely to a hypernephroid carcinoma was added by Schneider,[9] in 1956, and, most recently, Berg [10] cited a case of bronchogenic carcinoma with widespread metastases which involved a malignant hamartoma of the kidney. Thus, of the 19 cases reported of cancer metastasizing to cancer, a hypernephroma was the recipient malignancy in 13. The pertinent features of these and of two additional cases described herein are listed in the accompanying Table.

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Veterans' Administration Hospital, West Roxbury, Mass., and Department of Pathology, Harvard Medical School, Boston.

Report of Cases

CASE 1.—A 57-year-old white man was admitted to the Veterans' Administration Hospital, West Roxbury, Mass., for the first time in 1954, complaining of low back pain of five months' duration. There were no associated urinary symptoms, but the prostate was greatly enlarged, fixed, stony-hard, and nodular and was considered

Metastasis of Cancer to Cancer

Age	Race & Sex	Metastasizing Malignancy	Recipient Malignancy	Extent of Metastasis	Author
58	WM	Squamous carcinoma of lower jaw	Hypernephroma, right, nonmetastasizing	Local lymph nodes and recipient tumor	Berent, 1902[1]
41	WM	Carcinoma of thyroid	Hypernephroma, right, nonmetastasizing	Widespread	Hammann, 1927[2]
65	WF	Carcinoma of endometrium	Hypernephroma, left nonmetastasizing	Capillaries of recipient tumor	Walter, 1928[3]
	?	Carcinoma of lung	Hypernephroma, non-metastasizing	Widespread, only kidney metastasis in hypernephroma	Schmorl, 1928[4]
	M	Adenocarcinoma of prostate	Rhabdomyosarcoma (?) of mesentery, nonmetastasizing	Recipient tumor	Simard & Saucier, 1930[5]
47	WM	Carcinoma of lung	Hypernephroma, left, nonmetastasizing	Widespread, only kidney metastasis in hypernephroma	Walther, 1948[6]
66	WF	Adenocarcinoma of stomach	Hypernephroma, left, nonmetastasizing	Widespread, only kidney metastasis in hypernephroma	Walther, 1948[6]
58	WM	Carcinoma of pyriform sinus	Hypernephroma, left, nonmetastasizing	Widespread, only kidney metastasis in hypernephroma	Walther, 1948[6]
58	WF	Adenocarcinoma of thyroid	Adenocarcinoma of stomach, extent of growth not available	Widespread	Walther, cited by Rabson, 1954[7]
67	WM	Carcinoma of lung	Adenocarcinoma of prostate, extent of growth not available	Widespread	Walther, cited by Rabson, 1954[7]
55	WM	Carcinoma of lung	Adenocarcinoma of pancreas, extent of growth not available	Widespread	Walther, cited by Rabson, 1954[7]
64	WF	Melanoma of eye	Hypernephroma, left, nonmetastasizing	Widespread, only kidney metastasis in hypernephroma	Ortega et al., 1951[8]
72	WF	Adenocarcinoma of cecum	Preexistent lymphosarcoma at metastatic sites	Both widespread	Rabson et al., 1954[7]
58	WM	Carcinoma of lung	Hypernephroma, left, nonmetastasizing	Widespread, only kidney metastasis in hypernephroma	Rabson et al., 1954[7]
65	WM	Carcinoma of lung	Hypernephroma, left, nonmetastasizing	Recipient tumor & extension to epicardium	Rabson et al., 1954[7]
72	WM	Adenocarcinoma of prostate	Hypernephroma, right, with pulmonary metastases	Right kidney & recipient tumor	Rabson et al., 1954[7]
56	WM	Adenocarcinoma of prostate	Hypernephroma, right, nonmetastasizing	Widespread, only kidney metastasis in hypernephroma	Rabson et al., 1954[7]
60	WM	Adenocarcinoma of prostate	Hypernephroma, right, nonmetastasizing	Recipient tumor	Schneider, 1955[9]
59	WF	Carcinoma of lung	Angiolipomyosarcoma of left kidney, nonmetastasizing	Widespread, only kidney metastasis in recipient tumor	Berg, 1955[10]
57	WM	Adenocarcinoma of prostate	Hypernephroma, right, nonmetastasizing	Widespread, only kidney metastasis in hypernephroma	Present case
52	WF	Carcinoma of breast	Hypernephroma, left, nonmetastasizing	Widespread, only kidney metastasis in hypernephroma	Present case

carcinomatous. No biopsy was performed, since osteoblastic metastases were demonstrated radiographically throughout the vertebral column, pelvis, and bony thorax. Intravenous urography disclosed a filling defect in the right renal pelvis which was interpreted as a calculus. Acid phosphatase value was 10.2 units (King-Armstrong); alkaline phosphatase was 15 units (Bodansky). A bilateral orchiectomy was performed, and, with 5 mg. of diethylstilbestrol daily, all back pain disappeared within two weeks. After six months, the acid phosphatase was normal; the prostate was small and soft. One year later, progressive back pain and difficulty in voiding recurred subsequent to a lapse in estrogen therapy. Multiple painful nodules, varying from 1 to 3 cm. in diameter, appeared on the chest and abdominal wall. The prostate was again found to be markedly enlarged,

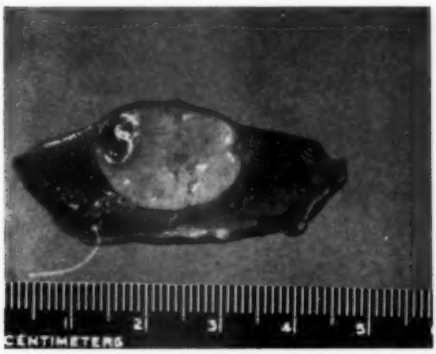

Fig. 1.—Representative segment of the renal tumor. Observe that it contains several nodules which are slightly darker in shade than the most peripheral portion of the tumor.

fixed, stony-hard, and nodular. There was rapid and progressive deterioration, with enlarging liver, anorexia, inanition and bronchopneumonia; death occurred two weeks after the final hospitalization and two years after the first symptom.

At autopsy the prostate was hard and asymmetric. Its right posterior lobe contained and was replaced by a poorly demarcated, hard, yellowish-gray mass, measuring 3.0 cm., which partially enveloped the seminal vesicles. There were metastases to regional lymph nodes, liver, lungs, adrenals, perirenal fat, pancreas, pleurae, pericardium, peritoneum, diaphragm, subcutaneous fat, ribs, and vertebrae. The right kidney weighed 175 gm. and measured 13×7×3.5 cm. A 2.0 cm. firm pale yellow tumor bulged from the upper pole anteriorly. The nodule was sharply demarcated from the renal cortex and was found, upon section, to be composed of yellow tissue peripherally and confluent glistening nodules of pale gray tissue centrally (Fig. 1).

Microscopically, a fairly well differentiated adenocarcinoma was present in the prostate. The renal tumor was composed of large pale well-demarcated cells, with foamy pale lipoid-like cytoplasm and moderately large dark nuclei. These cells occurred in small clusters, with intervening vascularized fibrous septa. Groups of smaller cells with uniform dark nuclei were dispersed throughout the renal tumor

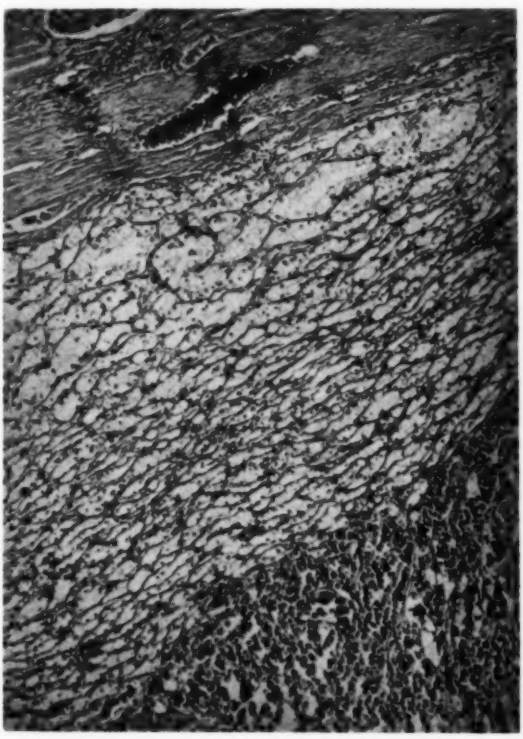

Fig. 2.—Three strata are apparent in this figure. The upper one consists of atrophic and scarred parenchyma which forms the tumor bed. The middle layer is representative of the renal tumor and illustrates both its adenomatous structure and the pallor of the cells composing it. The lowermost portion, representative of one of the nodules within the kidney tumor, has a poorly differentiated epithelial growth pattern which characterized the prostatic carcinoma in this case. Hematoxylin and eosin; ×60.

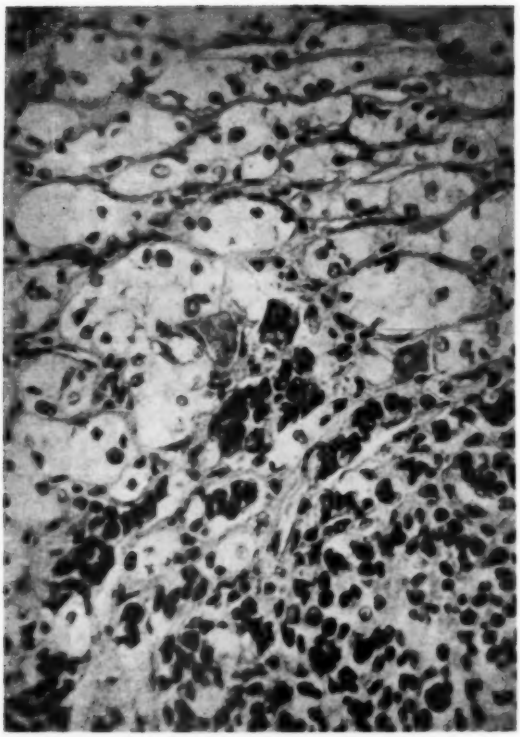

Fig. 3.—The small size, dark stain, and lack of organization of the tumor cells in the lower portion of the figure contrasts vividly with the structure of the host tumor. Hematoxylin and eosin; ×200.

(Figs. 2 and 3). The second type of cells was entirely different from the host tumor cells but identical with those of the prostatic tumor.

Comment.—This was a case of adenocarcinoma of the prostate with widespread metastases. One metastatic site was to another cancer, a hypernephroid or clear-cell adenocarcinoma of the right kidney.

CASE 2 (Provided by Dr. George Milles, Augustana Hospital, Chicago).—A 52-year-old white woman underwent a left radical mastectomy in 1948 at Augustana Hospital, Chicago. Two months prior to surgery she first noted a painful lump in her left breast. There was a hard mass which caused retraction of the overlying skin. The tumor proved to be an undifferentiated carcinoma which had involved the axillary nodes. The patient returned to the hospital in 1949 and in a week died as a result of widespread metastases. There were no urinary or kidney symptoms evident.

At autopsy the breast carcinoma had metastasized to the liver, mesentery of the small and large intestines, right and left adrenals, right auricle, ovaries, and vertebral bone marrow. The hilus of the left kidney was partially replaced by a 5×1.5×1.5 cm. soft, mushy, light grayish-tan tumor invading the renal veins.

Microscopically, the renal tumor was composed of rather large discrete cells with clear lipoid-filled cytoplasm and rather dark small nuclei. There was a scanty but vascular supporting stroma. Lying within the tumor there were groups of morphologically distinct anaplastic epithelial cells with much larger pleomorphic hyperchromatic nuclei, prominent nucleoli, and sparse basophilic cytoplasm. This second growth had the structure and appearance of the original breast cancer.

Comment.—This was a case of breast carcinoma treated unsuccessfully by radical mastectomy. At autopsy, the tumor was found to have spread widely, involving, among other structures, a hypernephroid carcinoma of the left kidney.

Comment

Metastasis of cancer to cancer is an exceedingly rare event, considering the frequency of neoplastic disease and the not uncommon occurrence of multiple malignancy.[11-13] Although the basis for this situation is conjectural, it seems reasonable to suggest that the requirements of a rapidly growing tumor are so great[14] that it provides an unfavorable environment for the implantation and development of a nutritively competitive cellular growth. Of the exceptions to this generalization, it is remarkable that hypernephroid carcinoma was the recipient neoplasms in more than two-thirds of the cases, since this is one of the less frequent forms of cancer. Although it is several times as common when there are multiple cancers, with an incidence in different series[11-13] varying from 2% to 5%, this still fails to explain the predominance of hypernephroid carcinomas over other cancers as a locus for metastatic growth. Moreover, in none of the series dealing with multiple cancer was there mention of metastatic deposits within a renal tumor, and so this occurrence, even among hypernephromas, is a rarity. In this collected series it is noteworthy that all except one (Rabson et al.[7]) was still largely confined to the limits of the organ. Perhaps this is a dormant form of cancer which, according to Warburg,[14] has not yet attained the degree of anaerobic metabolism which characterizes fully malignant growths. Since dormant growths have less stringent metabolic requirements than fully aggressive neoplasms, there would be less competition for essential nutrients and commensurately a greater likelihood for successful implantation and growth of a metastatic tumor. This presumably is the basis for the much commoner occurrence of metastases to benign tumors, as reported by Ortega, Li, and Shimkin.[8]

With a solitary exception (Berent[1]) all of the primary growths were widely disseminated, and it is reasonable to assume heavy and repeated exposure of the recipient tumor to lymphatic as well as vascular emboli. No single tumor seems to be more likely than others to metastasize to a hypernephroma when the factors of prevalence and anatomic position are considered. The lung was the primary site in four and the prostate, in four; varied origins accounted for the remaining seven.

Summary

Two rare cases of cancer metastasizing to cancer are reported. In each a widespread carcinoma originating in prostate and breast, respectively, formed secondary deposits in a localized hypernephroma. This sequence was true of more than two-thirds of all the reported cases. It was suggested that the success or failure of a secondary tumor to implant and grow depended upon competition with the host tumor for essential nutrients. The rarity of metastases to cancer would indicate that most often the supply of these substances is inadequate for the simultaneous support of two malignant growths. Localized hypernephroid tumors are considered dormant growths which have not yet attained the degree of anaerobic metabolism which characterizes fully malignant neoplasms. The smaller nutritive requirements of this form of tumor might well explain its relative frequency as the recipient neoplasm.

Laboratory Service, Veterans' Administration Hospital, Veterans of Foreign Wars Pkwy. (32).

REFERENCES

1. Berent, W.: Seltene Metastasenbildung. Zentralbl. allg. Path. 13:406-410, 1902.

2. Hammann, E.: Ungewöhnliche Metastasierung eines Sarkoms bei Bestehen zweier Tumoren, Frankfurt. Ztschr. Path. 35:256-264, 1927.

3. Walter, A.: Ein Fall von Metastasen des Uteruscarcinoms in ein Nierenhypernephrom, Ztschr. Krebsforsch. 27:451-456, 1928.

4. Schmorl, G.: Pathological Study of Schneeberg Lung Cancer, in Report of International Conference of Cancer, London, 1928, pp. 272-274.

5. Simard, C., and Saucier, J.: Métastase d'un épithélioma de la prostate dans un sarcome à

cellules géantes du mésentère, Bull. Assoc. franç. étude cancer 19:544-548, 1930.

6. Walther, H. E.: Krebsmetastasen, Basel, Benno, Schwabe & Co., 1948, p. 172.

7. Rabson, S. M.; Stier, P. L.; Baumgartner, J. C., and Rosenbaum, D.: Metastasis of Cancer to Cancer, Am. J. Clin. Path. 24:572-579, 1954.

8. Ortega, P., Jr.; Li, I. Y., and Shimkin, M.: Metastasis of Neoplasms to Other Neoplasms, Ann. West. Med. & Surg. 5:601-609, 1951.

9. Schneider, L. A.: Metastasis of Cancer to Cancer, Am. J. Clin. Path. 25:1288-1289, 1955.

10. Berg, J. W.: Angiolipomyosarcoma of Kidney (Malignant Hamartomatous Angiolipomyoma) in a Case with Solitary Metastasis from Bronchogenic Carcinoma, Cancer 8:759-763, 1955.

11. Warren, S., and Gates, O.: Multiple Primary Malignant Tumors: A Survey of the Literature and a Statistical Study, Am. J. Cancer 16:1358-1414, 1932.

12. Warren, S., and Ehrenreich, T.: Multiple Primary Malignant Tumors and Susceptibility to Cancer, Cancer Res. 4:554-570, 1944.

13. Watson, T. A.: Incidence of Multiple Cancer, Cancer 6:365-371, 1953.

14. Warburg, O.: On the Origin of Cancer Cells, Science 123:309-314, 1956.

A Method of Color Description for Use in Gross Pathology

An Adaptation of the ISCC-NBS Method of Designating Colors

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Descriptions of gross pathology specimens usually include at least three measurements: weight, dimensions, and color. The metric system easily and accurately measures the weight and the dimensions. In contrast, color descriptions are dependent upon each observer's personal view as to what constitutes a given color. The actual estimation of the color of a specimen varies from person to person, and frequently, the same observer will fluctuate in his appraisal of a color. In addition, color vocabularies vary, and unfamiliar color terms are often used.

This paper is an attempt to solve this problem by presenting a color chart which is based on the ISCC-NBS method of designating colors. Appel [1] urged adoption of this method for use in the field of dermatology. The Inter-Society Color Council,* in conjunction with the National Bureau of Standards, originally devised this method for use in the United States Pharmacopoeia and the National Formulary. This method provides for accurate color determinations couched in readily understood color terms and consists of a standard set of color names applied to the color chips of the "Munsell Book of Color." The National Bureau of Standards Circular 553,[2] "The ISCC-NBS Method of Designating Colors and a Dictionary of Color Names," issued Nov. 1, 1955, describes this method and provides the basic reference for the present paper.

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Veterans' Administration Hospital.

* The Inter-Society Color Council (ISCC) is formed of representatives from the major groups interested in color, as, the Federation of Paint and Varnish Production Club, the American Institute of Decorators, and the Society of Motion Picture and Television Engineers.

The Munsell Color System

The Munsell system consists of a systematic collection of color chips divided into 40 charts which comprise the "Munsell Book of Color." [3] The gradation between individual color chips is equal, so the Munsell system is referred to as a system of visually equispaced color scales.[4]

To understand the Munsell system, one should imagine a sphere with upper and lower poles and an equator (Fig. 1). The colors are packed into this sphere in an orderly fashion. The spectrum of colors is imagined wrapped around the equator. In the upper hemisphere the colors are light and the pole is white; in the lower hemi-

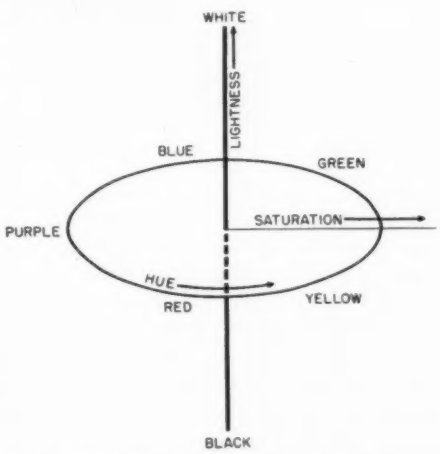


Fig. 1.—Dimensions of the color solid.

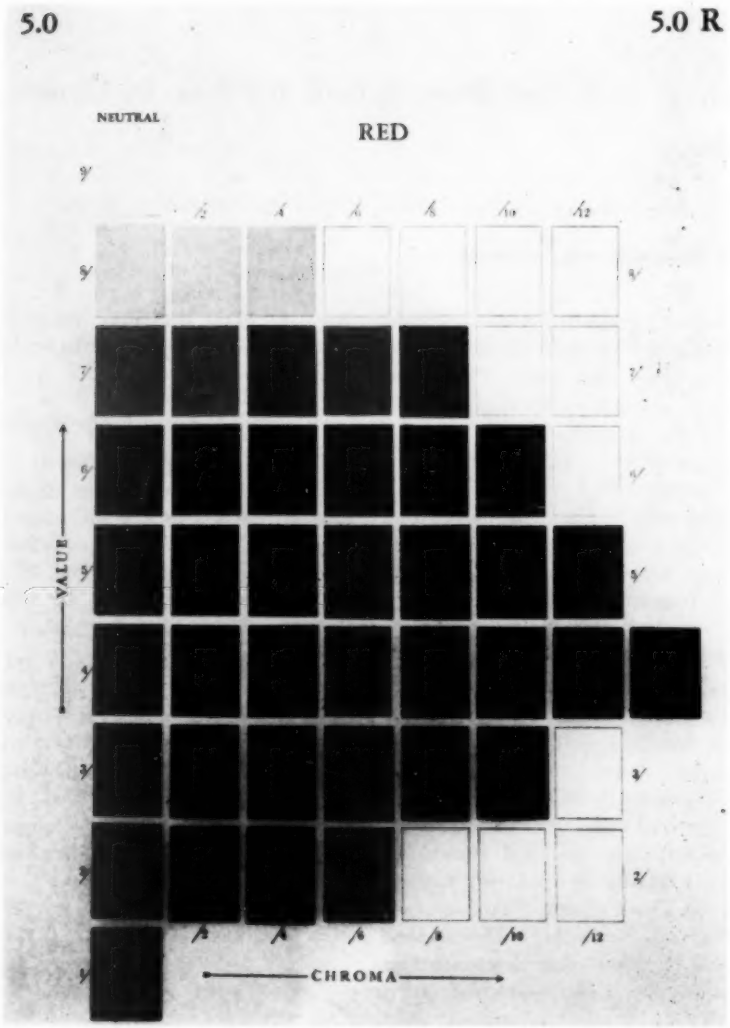


Fig. 2.—Constant hue chart 5.OR (library size) from the "Munsell Book of Color."

sphere the colors are dark and the pole is black. Shades of gray are arranged on a line which is stretched between the two poles and goes through the center of the sphere. As this line is approached from the surface of the sphere the colors become progressively more grayish. Therefore, the vivid or highly saturated colors are arranged on the surface of the sphere. This sphere, which is called a psychological color solid, is the basic concept behind the Munsell system and, therefore, the ISCC-NBS method of designating colors.

If a slice is made along a given longitude to the center of our imaginary sphere, the exposed section will be formed of merging colors. The grays will be toward the center, the dark colors toward the bottom, and so forth. Instead of merging colors, if this slice is formed of color chips which are visually equispaced, a chart from the "Mun-

TABLE 1.—*Abbreviations for the Hue Names Used in the ISCC-NBS System*

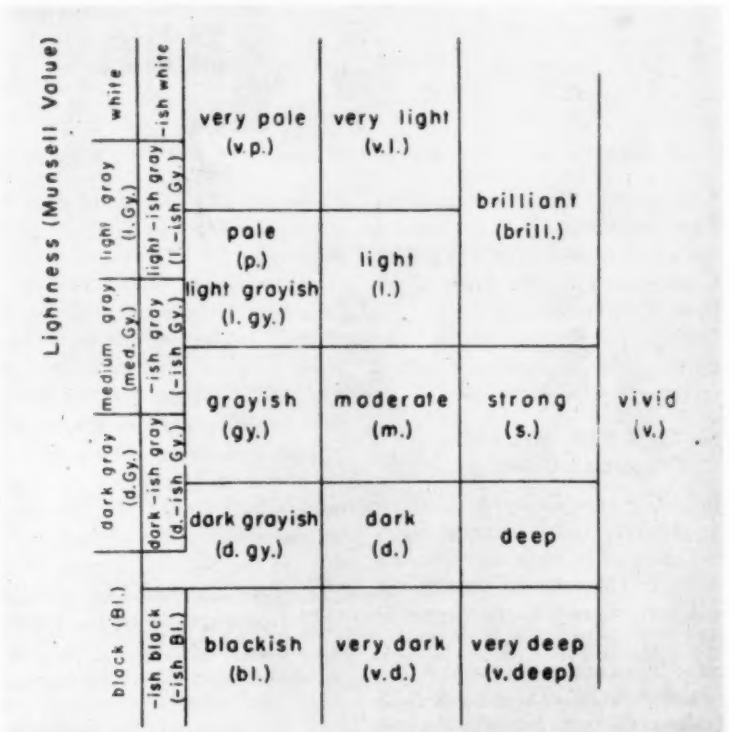
Name	Abbreviation	Name	Abbreviation
Red	R	Purple	P
Reddish orange	rO	Reddish purple	rP
Orange	O	Purplish red	pR
Orange yellow	OY	Purplish pink	pPk
Yellow	Y	Pink	Pk
Greenish yellow	gY	Yellowish pink	yPk
Yellow green	YG	Brownish pink	brPk
Yellowish green	yG	Brownish orange	brO
Green	G	Reddish brown	rBr
Bluish green	bG	Brown	Br
Greenish blue	gB	Yellowish brown	yBr
Blue	B	Olive brown	OlBr
Purplish blue	pB	Olive	Ol
Violet	V	Olive green	OlG

sell Book of Color" is produced (Fig. 2).† The "Munsell Book of Color" represents 40 such slices equispaced at nine-degree intervals.

The Munsell system is based on three attributes of colors: hue, value, and chroma. Hue refers to the color name, as red, yellow, or green. Value indicates lightness or darkness of a color, and the chroma indicates the saturation, or "strength," of a

† The charts are reproduced in black and white because color reproductions are usually inaccurate and unsuited for actual color measurements. Libraries usually carry copies of the "Munsell Book of Color," and traveling copies are available upon request from the Munsell Company.

Fig. 3.—ISCC-NBS scheme of hue modifiers.



Scheme of the hue modifiers, the "-ish" grays and the neutrals with their modifiers.
Abbreviations are given in parentheses.

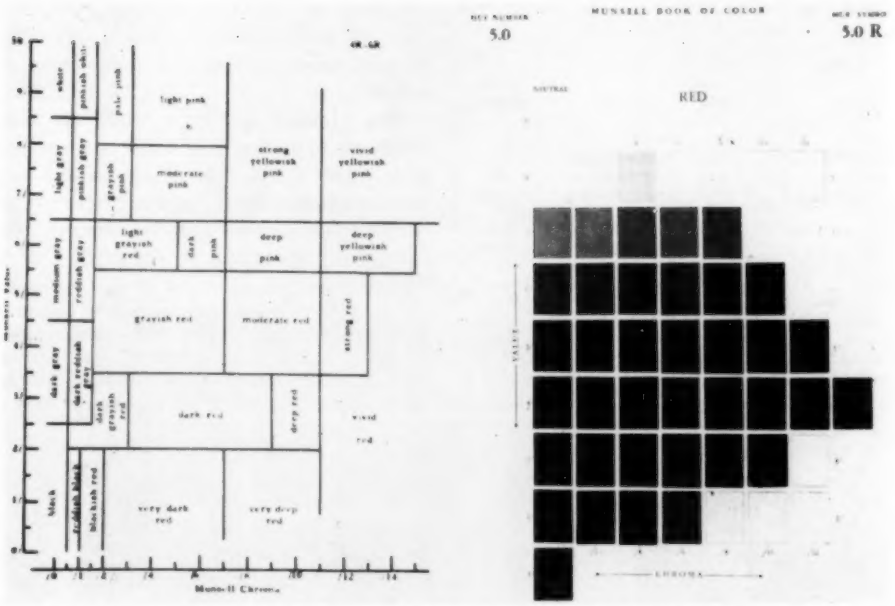


Fig. 4.—Constant hue chart 5.OR mounted with the appropriate ISCC-NBS color name chart.

color (Figs. 1 and 2). These three attributes provide the three-dimensional location of a color on the psychological color solid. This location is designated by the Munsell notation which is given numerically in the form of Hue Value/Chroma, or H V/C. The color chip at the far right of Figure 2 would be represented as 5R $^{4}/_{14}$.

The ISCC-NBS Method of Designated Colors

The Munsell system allows for extreme accuracy in matching and designating colors but requires the user to learn the numerical notation system. The value of the notation system is directly related to the degree of accuracy needed; for those who do not need a high degree of accuracy, the notation system is cumbersome. The ISCC-NBS method bridges the gap between the extremely accurate Munsell system and the loose usage of color terms by substituting a standardized list of popular color terms for the notation.

In this method descriptive terms are limited to 28 widely used color names or hue names (Table 1), and these hue names are supplemented by a system of hue modifiers (Fig. 3). No exotic or complicated color terms are used. Figures 1 and 2 demonstrate the relationship of the system of hue modifiers to the psychological color solid. Examples of color designations in the ISCC-NBS method are moderate red, light reddish-orange, or grayish-green.

The psychological color solid was parceled out to the various colors, and "maps" were made which limited the boundaries of the various colors. This part of the work was done in the National Bureau of Standards. The "maps" are found in the NBS Circular 553 [2] from pages 16 to 31. Figure 4 shows one of the Color Name Charts and the associated Munsell Hue Chart.

Materials and Methods

The basic equipment for matching colors is available, i.e., the "Munsell Book of Color" and the color name charts from the National Bureau of Standards Circular 553, which allow transposition of the Munsell notation to the proper ISCC-NBS designation.

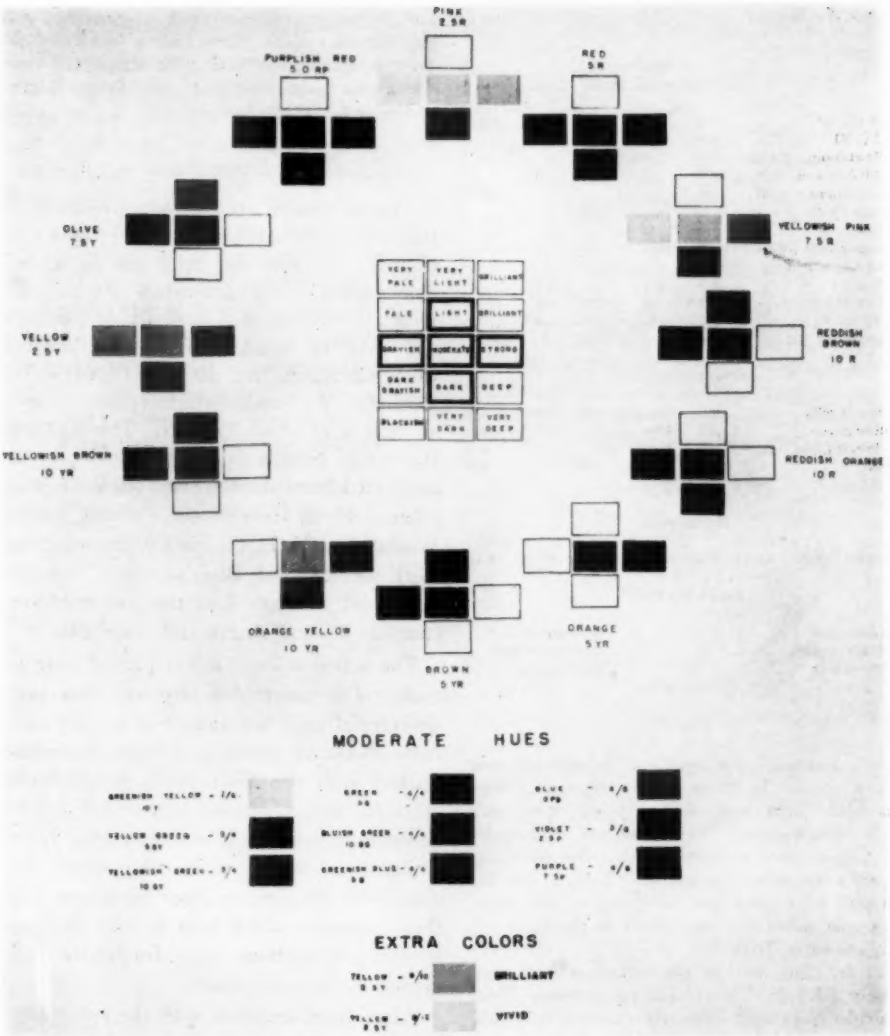


Fig. 5.—A color chart for use in pathology. The grayish, moderate, strong, light, and dark shades are represented by the circularly arranged crosses.

The first attempt to produce a practical color guide was to mount a number of Munsell color charts (library size) in a loose-leaf notebook. A photographed copy of the appropriated ISCC-NBS color name chart was mounted on the opposite page (Fig. 4). After matching the color, the proper ISCC-NBS designation was determined by referring across the page. It was soon discovered that, while accurate, this system was far too laborious, as the observer had to leaf through several pages of charts to make the color match and then refer across the page.

After abandoning this cumbersome system, effort was directed toward producing a single chart which would exhibit examples of most of the colors encountered in gross specimens and show the scheme for the hue modifiers (Fig. 5).

Of the 28 available ISCC-NBS hue names, 12, mainly in the red, pink, and orange ranges, were chosen. Single Munsell color chips were picked, guided by the ISCC-NBS color name charts, to represent the moderate, light, strong, dark, and grayish shades of these 12 hue names. These chips were arranged in a circle around a centrally

TABLE 2.—*Munsell Color Chips Used in Figure 5*

	Grayish	Moderate	Strong	Light	Dark
Pink (2.5 R)	7/2	7/6	7/8	--	6/6
Red (5 R)	4/4	4/8	4/12	--	2/6
Yellowish-pink (7.5 R)	7/2	7/6	7/8	--	6/6
Reddish-brown (10 R)	3/2	3/4	--	5/4	--
Reddish-orange (10 R)	5/6	5/8	--	--	4/8
Orange (5 YR)	--	6/8	6/12	--	--
Brown (5 YR)	3/2	3/4	--	5/4	--
Orange-yellow (10 YR)	--	7/6	7/10	8/6	5/6
Yellowish-brown (10 YR)	4/2	4/4	--	6/4	--
Yellow (2.5 Y)	7/4	7/6	7/10	8/6	6/6
Olive (7.5 Y)	4/2	4/4	--	6/4	--
Purplish-red (5.0 RP)	4/4	4/8	4/12	--	3/8

Moderate Hues Only

Greenish-yellow	10 Y	7/6	Greenish-blue	10 BG	4/4
Yellow-green	5 GY	5/4	Blue	5 PB	4/6
Yellowish-green	10 GY	5/4	Violet	2.5 P	3/6
Green	5 G	4/4	Purple	7.5 P	4/6
Bluish-green	10 BG	4/4			

Extra Colors

Brilliant yellow	2.5 Y	8/10	Vivid yellow	2.5 Y	8/12

Colors Not Used

Purplish-blue	Brownish-pink
Reddish-purple	Brownish-orange
Purplish-pink	Olive-brown
Olive-green	

placed scheme of hue modifiers. In addition, nine colors not in the range of the usual pathology descriptive area were shown by exhibiting only their moderate hues. The brilliant and vivid shades of yellow were included to describe more accurately the intimas of arteries. Table 2 lists the Munsell color chips used in preparing this chart. The nine colors not represented in the chart are also shown in Table 2.

In the chart most of the crosses are not completely filled in. This is for two reasons. First, the color chips must meet very exacting standards, and some chips are not available for technical reasons in manufacturing. Secondly, the ISCC-NBS nomenclature omits certain hue modifiers. For instance, there is no "light red" (Fig. 5). Instead, this area was termed "deep pink." These minor discrepancies are ignored in using the chart.

The color chart was mounted directly across from the gross dissecting table in the autopsy room. Color matches are made by comparing the sample directly with the chart.

Since this chart was designed to provide rapid color matches, extreme accuracy was sacrificed by using a limited number of color chips. The need of only a moderate amount of accuracy allowed for dispensing with several factors which are observed in making accurate color matches with the Munsell system. These include using daylight for the light source, having the sample the same size as the color chips, and using a neutral gray background.

Comment

Charts similar to the one prepared in this discussion can be assembled easily and cheaply. Its size and form can be varied quite easily. The individual color chips (library size—⅝ in.×⅞ in.) cost 20¢ each and may be obtained from the Munsell Color Company, Inc., 10 East Franklin St., Baltimore 2. Smaller chips (pocket size—½ in.×⅝ in.) cost 15¢ each. The National Bureau of Standards Circular 553 may be purchased from the Superintendent of Documents, U. S. Government Printing Office, Washington 25, D. C., for $2. For exacting work the "Munsell Book of Color" may be purchased. Volume I of the pocket edition contains 20 hue charts and costs $45.

The actual color matches proved easy to make. The major difficulty was that each observer already had notions as to what each color should be called, and these frequently clashed with the ISCC-NBS designations. It seems that a definite adjustment has to be made in converting to this system. Most pathologists have certain color terms for describing the various gross situations, and these patterns, which lead to easy and automatic descriptions, are frequently disrupted by the color chart.

After familiarization with the psychological color solid and the ISCC-NBS nomenclature, fairly accurate color descriptions can be made without referring to the color chart if the principles of hue, value, and chroma are kept in mind. After some exposure to the system, several autopsies were described without the direct aid of the chart. Afterward, the colors were matched with the "Munsell Book of Color." The results compared rather favorably, although there usually was some minor error in estimating the hue, value, or chroma.

Summary and Conclusions

The principles of the Munsell color system and the ISCC-NBS method of designating color are described briefly.

A color chart is presented which was designed for describing gross pathology specimens.

It is suggested that if the basic principles of the ISCC-NBS method and the psychological color solid are understood, color descriptions should become more accurate.

Adoption of the ISCC-NBS nomenclature will lead to uniformity in color descriptions.

Mr. W. Hale, Assistant Manager of the Munsell Color Company, Inc., gave permission for reproductions to be made from the "Munsell Book of Color." Mr. Hale gave advice during the entire course of preparing this paper. Mr. Kenneth Kelly, of the National Bureau of Standards, gave permission for use of illustrations from the National Bureau of Standards Circular 553 (Figures 1, 3, and 4 and Table 1). Miss Virginia Lerch drew Figure 5.

Veterans' Administration Hospital, University Drive.

REFERENCES

1. Appel, B.: Decadent Descriptions in Dermatology, Arch. Dermat. & Syph. 62:370-379, 1950.

2. Kelly, K. L., and Judd, D. B.: The ISCC-NBS Method of Designating Colors and a Dictionary of Color Names, U. S. National Bureau of Standards Circular 553, Nov. 1, 1955.

3. Munsell Book of Color, Defining, Explaining, and Illustrating the Fundamental Characteristics of Color, Ed. F, Baltimore, Munsell Color Company, Inc., 1929.

4. Nickerson, D.: Color and Its Description, Bull. Am. Ceramic Soc. 27:47-55, 1948.

Involvement of the Liver in Generalized Hypersensitivity Reaction

Report of a Case with Concomitant Multiple Microhamartomas of the Liver

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Involvement of the liver in generalized hypersensitivity reactions has been rarely described histologically, and little is known of the natural history of the hepatic lesions in such cases.[1-4] The present case provided an opportunity to study the liver in a biopsy specimen obtained during a drug reaction and at autopsy 15 days later.

Report of Case

The patient, a 78-year-old white man, was admitted to the Clinical Center of the National Institutes of Health on June 6, 1957, with a diagnosis of adenocarcinoma of the sigmoid colon. Prior to abdominoperineal resection of the colonic carcinoma, urinary obstruction was found, and a transurethral prostatic resection was performed on June 17, 1957. Recovery from the transurethral resection was uneventful, and he was given erythromycin from June 18 to June 23.

In preparation for the abdominoperineal resection of the carcinoma of the colon, he was given 12 gm. of succinylsulfathiazole (Sulfasuxidine) and 16 gm. of neomycin orally during the three-day period from June 23 to June 25. On June 24, his temperature rose to 39.8 C (103.6 F), with no clinical signs of localized infection. It was thought that he was having a febrile drug reaction, and the neomycin and succinylsulfathiazole were discontinued on June 26, 1957. On the following day he was afebrile and the abdominoperineal resection was performed.

At operation, the liver was found to be moderately enlarged, and the capsular surface was covered with many small white areas, some of which were slightly elevated. It was thought that these might represent metastases from the colonic carcinoma, and a biopsy specimen was obtained. A frozen section of the liver biopsy revealed no evidence of carcinoma, and a marked inflammatory-cell infiltrate was seen in the portal triads and in the small intrahepatic bile ducts in the triads. Since there had been no clinical evidence of cholangitis prior to operation, the extrahepatic biliary system was carefully examined by the surgeon. No gross evidence of cholangitis or obstruction of the extrahepatic bile ducts was found. The abdominoperineal resection of the colonic carcinoma was then performed.

Immediately postoperatively, the patient did well, and he was given penicillin and streptomycin. On the fourth postoperative day, he developed generalized pruritus and urticaria. Antibiotics were discontinued; diphenhydramine (Benadryl) was given, and the pruritus and urticaria rapidly subsided. After this episode, he became asymptomatic and was ambulatory. On the 15th postoperative day, he was found dead in bed. Clinically, it was thought that the cause of the sudden unexpected death was an acute myocardial infarct.

Pathologic Findings

Liver Biopsy (June 27, 1957).—Grossly, the wedge-shaped biopsy specimen of liver was reddish-brown, and the lobular architecture was preserved. There were several small gray areas 1 to 2 mm. in diameter on the capsular surface. Microscopically, there were two types of hepatic lesions present. One was a congenital anomaly of bile duct formation, with enlargement of portal tracts due to an increased number of small bile ducts surrounded by abundant fibrous tissue. These lesions are the so-called Meyenburg complexes, or multiple microhamartomas of the liver,[5,6] and are the small white areas which were suspected to be metastatic tumor at operation.

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National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare. Pathologic Anatomy Branch (Drs. Herrold and Rabson) and Surgery Branch (Dr. Smith), National Cancer Institute.

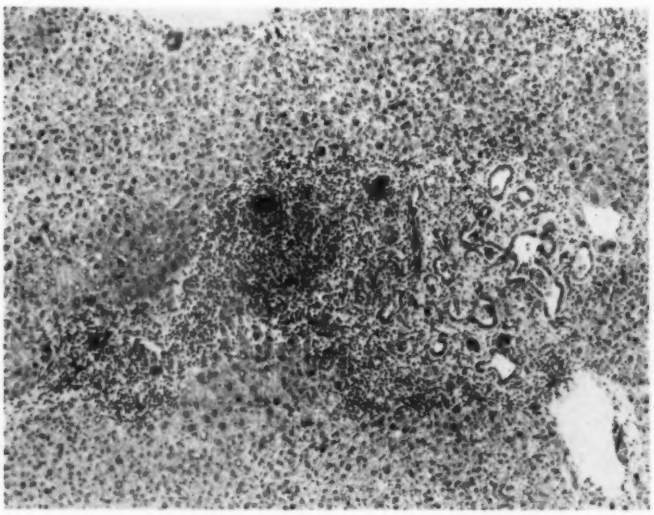

Fig. 1.—Liver biopsy specimen. There is extensive infiltration with eosinophils and other inflammatory cells in the portal tracts and an adjacent microhamartoma. Hematoxylin and eosin; reduced about 20% from mag. × 95.

The second lesion in the liver biopsy specimen consisted of a marked inflammatory-cell infiltrate in the portal tracts, with a predominance of eosinophilic leukocytes (Fig. 1). Large numbers of eosinophils were seen within the lumens of the small bile ducts, and eosinophils were also present in their walls (Fig. 2). Both perilobular and intralobular ductules were involved, and in some areas the inflammatory infiltrate extended into the hepatic parenchyma. Of special interest was the presence of a Meyenburg complex in which the inflammatory-cell infiltrate involved the stroma and the aberrant ducts of the malformation.

Abdominoperineal Resection Specimen (June 27, 1957).—The specimen consisted of anus, rectum, sigmoid colon, and a portion of descending colon. Two adenocarcinomas, one in the sigmoid colon and the other in the descending colon, were found. No metastases were seen in sections of 15 pericolic

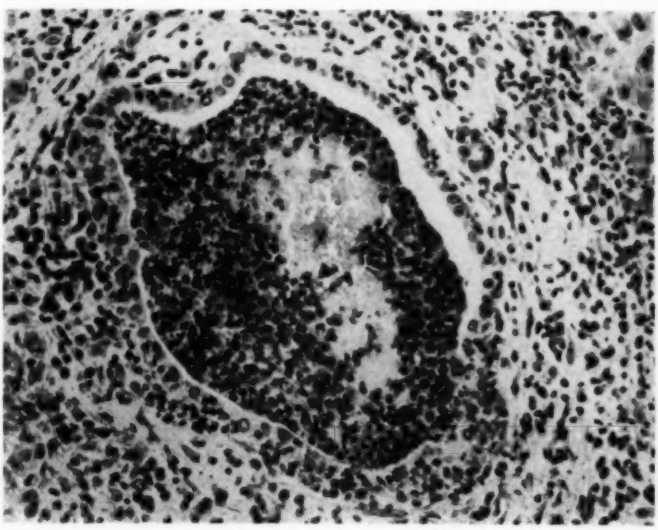

Fig. 2.—Liver biopsy specimen. Note the exudate of eosinophils in the lumen of an interlobular bile duct. Eosinophils also surround and infiltrate the wall of the duct. Hematoxylin and eosin; reduced about 20% from mag. × 275.

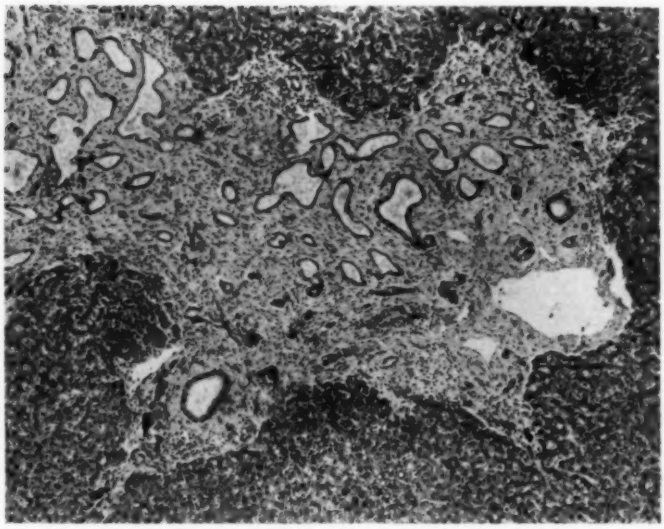

Fig. 3.—Liver at autopsy. There is almost complete resolution of the inflammatory-cell infiltrate in the portal tracts and adjacent microhamartoma. Hematoxylin and eosin; reduced about 20% from mag. × 80.

lymph nodes. Moderate to marked infiltration by eosinophilic leukocytes was found in all the tissues examined. A small granulomatous lesion with necrotic collagen and surrounding infiltration of eosinophils was seen in the pericolic fat.

Autopsy Findings (July 12, 1957).—The cause of death was a focal interstitial myocarditis. The cellular infiltrate was most marked in the ventricular septum and consisted primarily of eosinophils. No evidence of myocardial infarction was found.

In sections of the liver at autopsy, there was almost complete regression of the inflammatory process seen in the sections of the liver biopsy specimen 15 days before death (Figs. 3 and 4). Numerous microhamartomas were present, and these had only a minimal chronic inflammatory-cell infiltrate in their stroma in contrast to the

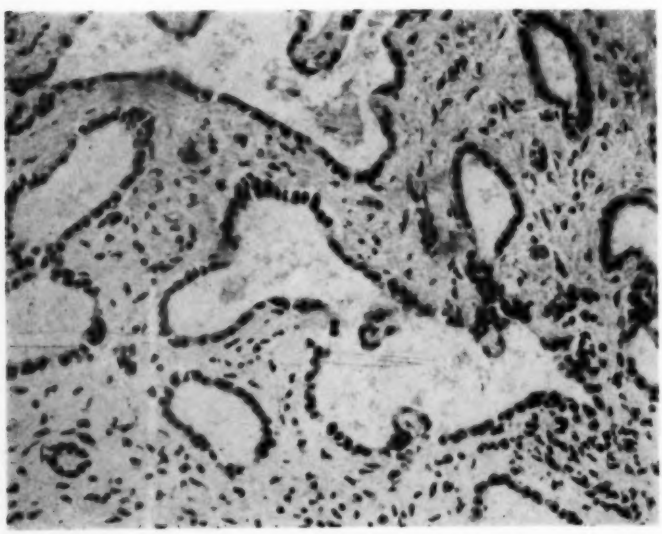

Fig. 4.—Liver at autopsy. Note the complete clearing of inflammatory-cell infiltrate in area of microhamartoma. Hematoxylin and eosin; reduced about 20% from mag. × 275.

marked infiltration with eosinophils seen in the biopsy specimen. Inspissated bile was present in the lumens of a number of small bile ducts in the portal tracts. No centrolobular cholestasis with bile plugs in the canaliculi was seen.

The gallbladder contained one calcified stone 1 cm. in diameter and five small pigmented stones 0.2 to 0.4 cm. in diameter. The entire extrahepatic biliary system was patent, with no evidence of constriction or obstruction. Microscopically, there was a mild chronic inflammatory-cell infiltrate in the wall of the gallbladder, and the common bile duct was free of inflammation.

The bone marrow was hypercellular, with an increased number of eosinophils.

Comment

The marked regression of the infiltration of the portal tracts by eosinophilic leukocytes in the 15-day interval between biopsy of the liver and death in this case is of great interest. Clinical observations of the transitory nature of hypersensitivity reactions are numerous, but histologic evidence of such changes in an internal organ is indeed rare.

The severity of involvment of the liver in this case with large numbers of eosinophils within the lumens and walls of intrahepatic bile ductules is remarkable. In view of the cholelithiasis found at autopsy, the possibility must be considered that the patient had an acute bacterial cholangitis at the time of the drug reaction, with the outpouring of large numbers of eosinophils in the exudate within the bile ductules. The absence of clinical evidence of cholangitis prior to surgery and the absence of gross evidence of inflammation of the extrahepatic biliary tract at the time of surgery would tend to make this interpretation unlikely. Cultures of the bile at operation were sterile. The clinical evidence of drug hypersensitivity, the diffuse infiltration of the pericolic tissues in the operative specimen by eosinophils, and the large number of eosinophils in the portal tracts of the liver in the biopsy specimen are all in accord with the diagnosis of generalized hypersensitivity reaction with hepatic involvement.

Recently, there has been considerable interest in drug reactions associated with jaundice and characterized histologically by bile stasis in the fine radicles of the intrahepatic biliary tract.[4] These reactions usually have been associated with administration of chlorpromazine or methyltestosterone, and neither of these drugs were given to our patient. It should be emphasized, in our case, that there was never any clinical evidence of jaundice and that the serum-bilirubin, cephalin-flocculation, and thymol-turbidity tests on the day after the liver biopsy specimen was obtained were all within normal limits. Unfortunately, no determinations of serum alkaline phosphatase were obtained during his course. Histologically, in spite of the severe inflammatory reaction in the portal tracts, centrolobular cholestasis with bile plugs in bile canaliculi, as usually described in chlorpromazine and methyltestosterone jaundice, was absent.

It is not possible, in the present case, to be certain of the specific drug which caused the hypersensitivity reaction. Both succinylsulfathiazole and neomycin were being administered at the time of the febrile reaction, and the fever rapidly subsided when these two drugs were discontinued. In view of the known association of sulfonamide drug reactions with hypersensitivity lesions in the liver,[7] it would seem most likely that small amounts of absorbed succinylsulfathiazole were responsible for the drug reaction in our patient.

Summary

A case of generalized hypersensitivity reaction with hepatic involvement is presented, and the histologic findings in the liver during the acute reaction and 15 days later are described. The liver also contained multiple microhamartomas which participated in the inflammatory reaction. The severe degree of involvement of the liver in this case was unusual, and the regression

of the inflammatory lesions after the 15-day interval was striking.

Mr. Gebhard Gsell, Pathological Technology Section, Laboratory of Pathology, National Cancer Institute provided the photomicrographs.

Pathologic Anatomy Department, National Institutes of Health (14).

REFERENCES

1. Popper, H.: Liver: Structure and Function, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1957, p. 658.

2. Días-Rivera, R. S.; Ramos-Morales, F., and Cintrón-Rivera, A. A.: Infiltrative Eosinophilia, A. M. A. Arch. Int. Med. 94:102-121, 1954.

3. Lusiani, G. B., and Sorcini, G.: Liver and Allergy, Minerva. med. 47:2024-2035, 1956.

4. Gutman, A. B.: Drug Reactions Characterized by Cholestasis Associated with Intrahepatic Biliary Tract Obstruction, Editorial, Am. J. Med. 23:841-845, 1957.

5. von Meyenburg, H.: Über die Cystenleber, Beitr. path. Anat. 64:477-532, 1918.

6. Popper,[1] p. 587.

7. French, A. J.: Hypersensitivity in the Pathogenesis of the Histopathologic Changes Associated with Sulfonamide Chemotherapy, Am. J. Path. 26:679-701, 1946.

Late Effects of Total-Body Roentgen Irradiation

III. Early Appearance of Neoplasms and Life-Shortening in Female Wistar Rats Surviving 1000 r Hypoxic Total-Body Irradiation

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Introduction

A single exposure to a large dose of total-body ionizing radiation has been considered a carcinogenic event by a number of investigators. Neoplasms have either appeared in greater numbers or developed sooner in irradiated animals, when compared to non-irradiated colonies of the same age.[1,2] Our own previously reported studies [3] have similarly shown an early onset of neoplasms, but the final incidence of tumors in our irradiated rats has been no higher than in the few very old rats of our control colony.

The present study provides our first opportunity, recently reported in a preliminary fashion,[4] to compare the final incidence of neoplasms in irradiated and control animals of identical stock when all members of both irradiated and control groups have been allowed to survive to a terminal state from spontaneous disease. Under this condition of comparison, a single large exposure to total-body x-radiation has proven an effective tumor-accelerating agent. The final total *incidence* of neoplasms developing during the shortened life span of the irradiated group *has not*, however, *been increased* by the total-body radiation exposure.

Major pathologic findings contributing to the death of the animals, as well as changes commonly associated with late irradiation damage such as epilation, skin ulcers, and cataracts, are also compared in the irradiated and control groups. The incidence of hypertension and nephrosclerosis in these same animals and their interrelation are the subjects of a separate report.[5]

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From the Departments of Pathology and Radiology and the Atomic Energy Project, University of California School of Medicine at Los Angeles.

This paper is based on work performed under Contract No. AT-04-1-Gen. 12 between the Atomic Energy Commission and the University of California at Los Angeles.

Methods and Materials

One hundred eighty-eight female Wistar rats obtained from Carworth Farms, weighing an average of 182 gm. with an estimated age of 4 months, were divided into four similar groups of near equal mean weight. The 100 rats of two of these groups each received 1000 r of total-body irradiation delivered from two simultaneously energized targets of a 250 kv. machine. Radiation factors were 15 ma., target distance 35 cm., field size 35 cm.^{2}, half-value layer 1.47 mm. of Cu, dose rate 170 r per minute. The animals were made hypoxic by ventilation of the plastic exposure cages with a 5% oxygen and 95% nitrogen mixture one minute prior to and during the radiation exposure, as previously described.[6] The 30-day L. D_{50} under these conditions of irradiation is between 1200 and 1400 r. After irradiation, rats were housed four to a cage and fed Rockland Rat Diet and tap water ad libitum. The animals were housed in a basement room without adequate temperature control during the first 15 months of this study. During winter months room temperature was probably consistently below that considered optimal. In addition, diurnal temperature fluctuations of 10 degrees (F) undoubtedly occurred. After 15 months the colony was moved to an air-conditioned vivarium. The other two groups consisting of 88 nonirradiated control rats were similarly housed and fed four to a cage. During the first 30 days post irradiation, 9 of the 100 irradiated animals died, and they were not included in the analysis of long-term radiation effects. The remaining 91 postirradiated animals surviving for longer than 30 days are a selected group to the extent that those few animals most susceptible to

acute radiation death had been eliminated from the study of delayed radiation effects.

Commencing one and one-half months and terminating eight and one-half months after irradiation, approximately one-half of the irradiated and control animals, respectively, were given a systematic course of prophylactic antibiotics consisting of alternating treatment with oxytetracycline (Terramycin) and penicillin and streptomycin, interspersed by rest intervals. In addition, weekly injections of oxophenarsine (Mapharsen) were administered during this period. Initially, polymyxin was included in the antibiotic plan, 0.5 mg. per animal. This dose was well tolerated in the controls, but unexpected death of six rats in the irradiated group after our first injection necessitated discontinuation of this drug. In addition, one irradiated and three control rats died within a few hours after injections of penicillin and streptomycin. These 10 animals, whose deaths were related temporarily to the use of antibiotics, were also deleted from the long-term survival statistics.

After seven months of systematic antibiotic therapy, this program was discontinued. From this point, 8½ months post irradiation, to the death of the animals, use of antibiotics was limited to one three-day course of penicillin and streptomycin 17 months post irradiation in all the surviving irradiated animals and two three-day courses of penicillin and streptomycin in the surviving control animals 26½ months and 28 months after the time of irradiation.

Indirect blood pressure measurements were performed in all rats at approximately monthly intervals, commencing 13 months post irradiation. The tail-cuff acoustic method of Friedman and Freed[7] was used with light Myotal* anesthesia, as detailed in a separate communication.[8] One irradiated and one control rat died shortly after one of these anesthetic procedures. These two animals were also discarded from the long-term survival study.

The initial distribution of rats in the irradiated and control groups is shown in Table 1. Deleting deaths attributable to acute radiation illness, use of antibiotics, and anesthesia during blood pressure determinations leaves a residual of 83 irradiated and 84 control rats which were observed until death occurred or was imminent from spontaneous causes. These 167 rats provide data on post-irradiation longevity, body weight, and the incidence of cataracts.

During the three-year period of this study, deficiencies in our observation schedule on weekends or holidays and the inevitable cannibalism associated with housing rats four to a cage reduced the number of autopsies to a total of 61 in the irradiated group and 64 in the controls, as shown in the bottom line of Table 1. These 125 autopsied animals are the basis for our analysis of the incidence of neoplasms and other diseases. Autopsies included microscopic examination of all major organs, including the central nervous system. All neoplasms recorded are confirmed by microscopic evaluation. The use of prophylactic antibiotics during the early months of the study did not apparently alter either final mortality or the development of disease in either irradiated or control groups, and so no further subdivision on this basis is made in subsequent analyses.

In all charts and tables duration of survival of both irradiated and nonirradiated control groups is computed from the date of radiation exposure. All rats were approximately 4 months old at this arbitrary starting point.

* Stated to consist of pentobarbitol sodium with mephenesin.

TABLE 1.—*Selection of Animals Observed Throughout Life Span from Initial Group of Irradiated and Control Rats*

	1000 r			Control		
	Prophylactic Antibiotics			Prophylactic Antibiotics		
	Yes	No	Total	Yes	No	Total
Initial No. of rats	49	51	100	44	44	88
30-day postirradiation deaths	3	6	9	--	--	--
Antibiotic deaths	7	--	7	3	--	3
Anesthetic deaths	0	1	1	0	1	1
Rats observed through life span	39	44	83	41	43	84
Autopsies performed	28	33	61	33	31	64

Observations

Survival.—Survival of the 167 postirradiated and control animals observed throughout their life span is shown in Chart 1. Rats receiving prophylactic antibiotics are separately plotted. During the seven-month period of this systematic antibiotic therapy, no deaths from spontaneous disease were recorded in either antibiotic-treated irradiated or control groups. The first two deaths in the antibiotic-treated animals occurred at eight and one-half months. At this time the antibiotic-treated animals of both the control and the irradiated groups were losing weight and appeared generally in poorer condition than their nontreated counterparts, and so this prophylactic antibiotic regimen was discontinued. This seven-month period of antibiotic therapy in one-half of both the

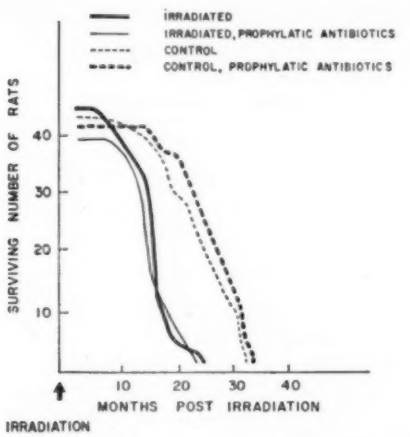

Chart 1.—Survival of control and irradiated Wistar rats following 1000 r hypoxic total-body irradiation.

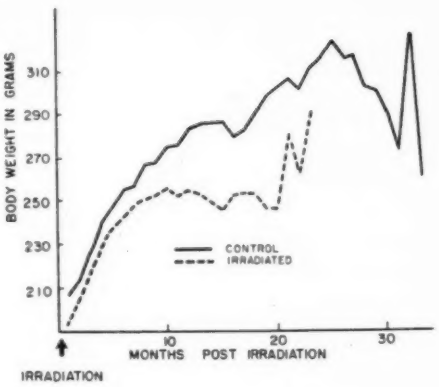

Chart 2.—Mean total body weight of surviving control and irradiated female Wistar rats at monthly intervals after date of irradiation.

irradiated and control rats may have postponed a few deaths during the course of therapy, as suggested by the flat curves of the treated groups during this period. The similar shapes of the survival curves after the prophylactic antibiotics were discontinued indicates, however, no residual or delayed influence of this antibiotic therapy on long-term survival of either the controls or irradiated animals.

All irradiated animals were dead by 24 months post irradiation. The last control rat died 33½ months after the date of irradiation, with an actual age of 37½ months. The 15-month mean postirradiation survival of the 83 irradiated animals represents a 39% shortening of postirradiation life span when compared with the 24½-month mean survival of the controls.

The shapes of the irradiated and control survival curves based upon the smaller number of 125 autopsied animals are similar to those of Chart 1, which is derived from all 167 rats of the entire long-term study. Mean survivals of the autopsied animals were 17 and 26 months, respectively, for the irradiated and controls. This similarity of mortality statistics from the autopsied animals and the total groups suggests that conclusions based upon autopsy data may be safely applied to the groups as a whole without serious error.

Mean Total Body Weights.—Mean total body weights of the surviving rats of both control and irradiated groups are plotted in Chart 2. One month post irradiation the irradiated animals had already recovered from the acute radiation reaction, as shown by a weight gain of 12.0 gm. above the preradiation level of 182 gm. The irradiated group, although showing a satisfactory rate of gain for the next seven or eight months, nevertheless failed at any time to reach the mean size of the nonirradiated controls. After 10 months a gradual weight decline occurred during the same months when an increase in mortality in the radiation group was observed. This decline in mean weight of the irradiated group after 10 months is not caused by selective death of the larger animals of this group. The mean curve is representative of the weight curve of the average individual irradiated rat. The erratic portions of both curves at the far right of Chart 2 are obviously based on progressively declining numbers of surviving animals and are of doubtful significance.

Cataracts.—Time of appearance of cataracts was recorded in clinical records in all 167 animals of the long-term study. Every rat received 1000 r radiation and surviving

TABLE 2.—*Common Major Pathologic Findings at Autopsy in Irradiated and Control Wistar Rats*

	Survival Time After Onset of Study, Mo.	Total No. Rats	Epilation	Skin Ulcers	Large Benign Tumors	Malignant Tumors	Pituitary Adenomas	Acute Inflammation		Nephrosclerosis	Cause of Death Uncertain
								Thorax	Abdomen		
Irradiated	2-24	61	33	36	11	8	0	38	23	18	8
Unirradiated controls	2-24	22	4	9	2	6	3	11	1	0	2
	25-33½	42	40	36	16	10	10	28	18	0	1
	2-33½	64	44	43	18	16	13	39	19	0	3

at least nine and one-half months thereafter eventually developed at least one cataract, as determined by gross inspection. The first cataract of the irradiated group was observed seven and one-half months post irradiation. The mean appearance time was 10½ months. An unexpected high incidence of cataracts also appeared in the very old control animals. All 14 of the rats surviving 31 months after the time of radiation also possessed cataracts, the first of these appearing at 26½ months. The time of appearance of cataracts is closely correlated with the period of mean weight decline in both the irradiated and the control animals.

Major Pathologic Findings at Autopsy.—A tabulation of the commoner major autopsy findings contributing to the death of the 125 autopsied animals is shown in Table 2. The incidence of epilation and skin ulcers is also included.

Not all benign and malignant tumors are tabulated as major findings. Benign extracranial tumors are listed only when the size of the neoplasm was estimated to be a major hindrance to the mobility, function, or nutrition of the animal. The pituitary adenomas listed as major findings were estimated from their size to have contributed to an increase of intracranial pressure. Malignant tumors have been tabulated as a major finding only when evidence of dissemination was found. Small microscopic growths, even though histologically malignant, were not considered to contribute significantly to the death of the animal.

This colony of rats has also shown the usual high incidence of acute and chronic inflammatory diseases, presumably of infectious origin so common in this species. The use of antibiotics during the early period of the study produced no significant reduction of these diseases, as estimated by extent of microscopic alterations in the treated half of the colony. The lungs particularly are the seat of chronic peribronchial inflammatory infiltrations which become progressively severer with increasing age. In addition, bronchiectasis, sometimes so severe as to convert an entire lobe of a lung to a solitary pus-filled cyst-like structure, is common. Acute pneumonia with pleurisy and acute purulent pericarditis are also common autopsy findings.

Below the diaphragm the female genital tract is the principle locus of acute inflammatory disease. Occasionally, acute purulent peritonitis, acute enteritis, and acute pyelonephritis are also observed.

Purulent middle-ear infections are also common, but acute meningitis, secondary to otitis media, accounts for death in only two cases.

The principle loci of these inflammatory diseases have been grouped for purposes of comparison into those within the thorax and those below the diaphragm.

Nephrosclerosis,[3,8] as classified here, is a severe bilateral diffuse disease. Glomerular capillary tufts and arterioles are greatly thickened with a periodic acid-Schiff-positive material. The cortex is extensively occupied by dilated tubules containing large hyaline casts. Inflammatory-cell infiltrate is minimal. This generalized process can be distinguished from the small focal scars so common in kidneys of old rats.[3]

TABLE 3.—*Tumor Incidence in Female Wistar Rats After 1000 r Total-Body X-Irradiation*

	Postirradiation Survival, Mo.							
	2-6	7-12	13-18	19-24	25-30	30-33½	Total	%
Deaths, No.	1	1	43	16			61	
Rats with tumors	0	0	20	9			29	48
Benign			16	8			24	39
Malignant			6	5			11	18
Multiple			4	5			9	15

Control animals are subdivided into those dying during the first 24 months after the onset of the experiment and those living longer than 2 years, so that a comparison of prevalence of disease may be made between irradiated and control animals of the same chronologic age. As seen in the first two lines of Table 2, epilation, large benign tumors, intra-abdominal inflammations, and nephrosclerosis show a higher incidence in the irradiated group than in the controls of the same age. On the other hand, a larger proportion of these younger control rats have malignant tumors, although a greater number of malignant neoplasms are observed in the irradiated animal. The absence of large chromophobe adenomas of the pituitary in the irradiated group is conspicuous.

With the exception of nephrosclerosis, disease prevalence in the older control rats of the 25- to 33½-month survival period is comparable to, or in excess of, that observed in the irradiated group. Nephrosclerosis with the histologic features previously described [3,8] is substantially increased in final incidence in the irradiated animals. This particular type of nephrosclerosis was not observed in this group of control rats, even among those reaching advanced age. We have, however, been able to produce nephrosclerosis indistinguishable by usual histologic techniques from that observed in these irradiated animals in nonirradiated rats made hypertensive by other means.[5]

In this entire autopsy study no lesion that uniquely defines the end-results of total-body radiation exposure has been detected. In general, those pathologic changes are found in the irradiated animals that, in time, affect the nonirradiated to the same or greater degree. An exception to this latter generalization would appear to be the high incidence of nephrosclerosis in the irradiated animal and certain changes in the incidence of specific tumor types to be outlined in the following sections.

Incidence of Tumors.—The occurrence of all tumors in irradiated and control rats dying during six-month intervals of post-irradiation survival are shown in Tables 3 and 4. The large number of deaths in the irradiated group during the 13- to 18-month survival period is accompanied by a high tumor incidence. The presence of many benign and multiple tumors during this period is in contrast to the control group dying during the same period when only malignant tumors were observed. The total incidence of tumor-bearing animals (three in the small control group of seven) is, neverthe-

TABLE 4.—*Tumor Incidence in Control Female Rats* *

	Survival After Onset of Study, Mo.							
	2-6	7-12	13-18	19-24	25-30	30-33½	Total	%
Deaths, No.	1		7	14	22	20	64	
Rats with tumors	0		3	10	20	17	50	78
Benign			0	9	16	16	41	64
Malignant			3	3	7	3	16	25
Multiple				3	9	12	24	37

* Of similar age to those in Table 3.

TABLE 5.—*Neoplasms in Irradiated and Control Female Wistar Rats*

Organs	Tumor Types	Irradiation	Control
Breast	Fibroadenoma	14	34
	Carcinoma	1	
Ovary	Benign	8	3
	Malignant	1	
Uterus	Benign	1	
	Malignant		3
Adrenal	Adenoma	2	
	Carcinoma		1
Pituitary	Adenoma	1	21
Pancreas	Adenoma acinus	1	
	Adenoma islet cell		1
Kidney	Adenoma	2	
	Sarcoma		1
Lung	Malignant	5	6
Bone	Sarcoma	1	
Skin	Mixed tumor	1	
	Carcinoma		1
Pudendal gland	Adenoma		1
Intestine	Leiomyoma		1
Liver	Adenoma	1	1
Disseminated	Malignant	4	1
Lymphoid tissue	Lymphoma		4
Brain	Glioma		1
Mouth	Adenocarcinoma		1
Total No. of Tumors		43	81
Benign		31	63
Malignant		12	18
Total No. of Rats		61	64

less, comparable to the larger irradiated group. In the 19- to 24-month survival period, where approximately the same number of deaths occurred in both irradiated and control groups, the incidence of tumors and their distribution between benign, malignant, and multiple are very similar.

In the later periods, 25 to 30 and 30 to 33½ months, when all irradiated rats are already dead, a very high incidence of neoplasms is observed in the control group. The large number of deaths in these later periods in this series, where all control animals were allowed to survive until spontaneous termination, is in contrast to the very few older control animals in our studies previously reported.[3] This older age distribution in our present control series, with the very high tumor incidence during the later periods of life, heavily outweighs the accelerated appearance of neoplasms in the younger irradiated animals. The final incidence of tumors of all three classifications, benign, malignant, and multiple, are substantially higher in the control series.

Distribution of Tumor Types in Irradiated and Control Groups.—A list of tumor types found in the irradiated and control rats comprises Table 5. Benign tumors of the breast, ovary, pituitary, and liver and malignant tumors of the lung were found in both irradiated and control groups. Pituitary and breast tumors were more frequent in the control group, whereas ovarian tumors were more prevalent in the irradiated animals. The incidence of these latter three neoplasms in the various postirradiation time periods is further shown in Table 6. The remaining neoplasms listed in Table 5 are derived from a large number of tissues about equally dispersed among the two groups of animals. This tendency for a very large variety of neoplasms to appear in older control animals suggests caution in attributing any single neoplasm observed after total-body irradia-

TABLE 6.—*Incidence of Neoplasms Most Frequently Observed at Autopsy During Several Periods of Postirradiation Survival and in Control Animals of Same Age*

	Postirradiation Survival, Mo.*									
	12-18		19-24		25-30		30-33½		Total	
	I	C	I	C	I	C	I	C	I	C
Breast, fibroadenoma	12		2	3		15		16	14	34
Ovary, benign	6		2	1				2	8	3
Pituitary, adenoma			1	7		7		7	1	21
Lung, malignant	2	2	3	1		2		1	5	6
Lymphoma				1		1		2	0	4
Deaths, No.	43	7	16	14		22		20	59	63

* I indicates irradiation; C, control.

tion to the effects of the radiation exposure. Whereas it is true that 8 tumor types were found in the irradiated group and not in the controls, 10 tumor types were found in the controls but not in the irradiated animals.

The summary of all tumors observed in this study at the bottom of Table 5 reveals twice as many benign tumors in the 64 control animals as were found in the 61 irradiated rats. Malignant tumors were also more frequent in the control group, although not to the same degree. Four of the eighteen malignant tumors of the control group were lymphomas, a tumor type which did not appear in the irradiated rats.

Table 6 shows the time distribution of four of the five tumor types prevalent in both control and irradiated animals. Lymphomas, although not found in the irradiated group of this study, are also included.

The early onset of breast neoplasms in the irradiated animals is clearly shown, although the final incidence favors the control group two to one. The early appearance of ovarian tumors in the irradiated rats is also again present, although, in this series, ovarian tumors have also been found in the control group, in contrast to their absence in the controls of our previous series.[3] Ovarian tumors do, however, appear to be specifically increased in this species by total-body irradiation at this dose level, as previously reported.[3] The well-known high incidence of nonfunctioning pituitary adenomas in older rats is again evident here, but their near absence in the irradiated group is difficult to explain. The younger age at death in the irradiated group cannot entirely account for this low incidence. In the 19- to 24-month survival period, 7 of the 14 control rats already possessed this neoplasm, while only 1 of the 16 irradiated rats was so afflicted. Malignant tumors of the lung were thought possibly to be increased in numbers in our previous study.[3] Now, in the presence of more older control material, lung malignancies appear sooner but not in greater final numbers.

Lymphoma is predominately a disease of the old rats in this series. Only one of the four examples of this disease in the controls appeared within the survival period of 19 to 24 months, when some of the irradiated animals were still alive. The onset of lymphoma does *not* appear to be accelerated by the radiation exposure used in this study in the adult female of this Wistar rat strain.

Organ Weights.—Pituitaries of the control and irradiated animals are approximately the same weight when expressed as wet weight per unit of total body weight. No difference in pituitary weights between those animals, with or without neoplasms, is observed. Many large mammary glands were observed in both the irradiated and the control groups, which, on histologic analysis, proved to be hyperplastic secreting breast tissue, rather than neoplasm. No relationship between the presence of secreting breast tissue and pituitary tumors in the control animals, or pituitary size in either irradiated or control rats, is observed.

Control rats, two years after the date of radiation, tend to possess larger adrenal glands expressed both as wet weight or wet weight per kilogram of total body weight.[5] Since almost all of these old control rats have neoplasms, the relation of adrenal size to presence or absence of neoplasms could not be evaluated in this group. In the irradiated rats, no difference is observed in adrenal size in the presence or absence of neoplasms. As previously reported, many of the irradiated rats, regardless of length of postirradiation survival, possess adrenal glands showing focal cortical fatty changes.[3] This lesion is also common in control rats surviving at least 27 months after the date of radiation.

Liver weights of both irradiated and control rats are remarkably constant when expressed as wet weight per unit of body weight. This relationship holds true even in the presence of very large neoplasms of the breast weighing as much as one-third of the total weight of the animal.

Heart and kidneys of the irradiated animals are again larger than these organs in

the controls of the same age.[3] The significance of these heart and kidney weight changes are discussed in a separte communication concerned with hypertension in these same animals.[5]

Comment

The reduction of life span following total-body radiation exposure has been analyzed by Blair,[9] utilizing published data on the mouse and rat. He concludes that the life-span-shortening effect of a single total-body x-ray exposure is proportional to the size of the radiation dose up to about two-thirds of the L. D.$_{50}$. When this straight line relationship is projected to the L. D.$_{50}$ dose ordinate, a life-span shortening of approximately 23% is indicated. The 30-day L. D.$_{50}$ in our Wistar rats for the conditions of hypoxic radiation exposure used here is not precisely defined by our earlier data but falls between 1200 and 1400 r.[6] The 1000 r currently employed then represents somewhere between 71% and 83% of an L. D.$_{50}$ dose under these conditions. This dose is greater than the two-thirds of an L. D.$_{50}$ estimated by Blair to be the upper limit of the straight-line longevity-dose relationship, and our observed life-span shortening of 38.8% is somewhat greater than the figure extrapolated from Blair's extended curve. The 38.8% shortening observed in this study following our dose of 71% to 83% of an L. D.$_{50}$ falls closer to the curve equating life shortening with larger single radiation doses based upon Furth's data in male LAF_1 mice,[10] as also analyzed by Blair.[9] The shortening of life span reported here is then in general agreement with that observed by others when the dose is expressed as a percentage of L. D.$_{50}$, notwithstanding the use of hypoxic hypoxia in these studies to protect against the acute effects of the radiation exposure. Whereas the hypoxic state allows the rat to survive a substantially larger absolute radiation exposure, the relationship of dose, expressed as per cent of L. D.$_{50}$, to reduced longevity remains essentially unchanged. In our laboratory the 30-day L. D.$_{50}$ total-body radiation dose for adult female Wistar rats delivered under normal atmospheric conditions is slightly in excess of 600 r.[6] The 1000 r hypoxic dose used in this study would then be comparable in its effect on longevity to 71% to 83% of this L. D.$_{50}$ in air, or approximately 425 to 500 r.

This dose-longevity relationship suggests that the mechanisms of protection involved in the reduced oxygen tension state is operative upon both the remedial and permanent components of radiation-induced injury,[11] assuming that reduction of life span is a measure of this nonreversible component. Other stigmata of permanent radiation damage in the rat, such as incidence of hypertension and nephrosclerosis and incidence and appearance time of ovarian and other tumors, must also be analyzed before reduction of life span can be accepted as a measure of all late radiation effects. The very few rats of our earlier data[3] available for comparison after a 500 r exposure in either 20% or 5% oxygen environment do not show a striking difference in the incidence of tumors or nephrosclerosis.

Complete autopsies in 125 of the 167 rats of this study, with histologic evaluation of all major tissues, assist in evaluating the reason for the reduced longevity in the irradiated animals. Does the early appearance of neoplasms, for example, or some other specific disease alone account for the reduced life span? Or do all the diseases prevalent in old rats appear sooner in these postirradiated animals with the retention of the relative incidence of all diseases characteristic of old age? In the latter proposition, the early appearance of neoplasms in the irradiated animals would be only a part of what might be called an accelerated aging process, a concept which has been supported by Blair[12] and others.

In general, a comparison of the major pathologic findings in the irradiated group with the old control rats of the 25- to 33-month survival group in Table 2 shows a very similar spectrum of disease processes, supporting this concept of accelerated aging. Only nephrosclerosis stands out as a patho-

logic change, common in the irradiated group but rare in the controls at any age. In contrast, advanced extracranial neoplasms, both benign and malignant, are more prevalent in both the aged controls and the control group as a whole than in the irradiated group of animals. In this Wistar rat strain the early onset of neoplasms should not be singled out as a disproportionate explanation of the reduced longevity following radiation. The proportion of deaths attributable to tumors in the irradiated series is no greater than exists in the control group of similar age. This observation, of course, does not refute the clinical and pathological findings in the individual case, indicating that some irradiated rats of this study *did* die at an early age as a consequence of their accelerated neoplasms.

Exceptions to this generalization concerning the accelerated onset of tumors as a part of early aging are apparent when individual tumor types are studied. The present study adds further evidence that ovarian tumors are specifically increased in frequency in the rat after total-body irradiation, extending an observation previously made in the mouse.[1] In contrast, the incidence of pituitary adenomas in the irradiated animal is decreased in this study; nor can this lower incidence of pituitary tumors entirely be explained by the shortened life span of the irradiated animals. In the 19- to 24-month survival period, animals of the same age are compared. Pituitary tumors favor the controls seven to one. This alteration of the incidence of particular tumor types, both derived from endocrine glands, leads to the speculation that some form of endocrine related disturbance in metabolism is a part of this late radiation effects syndrome.

Among the 39 neoplasms of known origin observed in the irradiated rats of this study, 29 were derived from organs with known endocrine secretions or from endocrine target organs with reproductive functions. Likewise, 64 of the 80 tumors with established origin in the controls were similarly located. Slightly over one-half of these tumors were in sex-hormone target organs.

The predominance of endocrine-associated tumors in both irradiated and control rats emphasizes that irradiation has not altered the predilection of certain tissues for neoplastic growth. This predilection is, no doubt, strongly influenced by genetic factors in the inbred rat, just as it is in the inbred strain of mouse.

In the Wistar strain of rats used in these studies, there is presently no evidence that those tissues known to be most seriously damaged by acute radiation exposure are selectively involved in the tumor-accelerating process that follows radiation doses of the magnitude used here. Tumors of the lymphatic system, bone marrow, and gastrointestinal tract are all rare.

Whether the tumor-accelerating influence of the ionizing radiation operates directly upon the individual tissues involved in the neoplastic process, or whether the total tumor controlling mechanism of the entire organism is altered by the widespread biophysical insult, cannot be settled by these data. It is hoped that long-term studies in progress involving the incidence and location of tumors in rats having selected portions of the body shielded from direct radiation exposure will help resolve this problem.

The well-known direct-radiation tumor inciting effects of large doses of external radiation upon skin, bone, and other tissues cannot be disregarded. On the other hand, an indirect mechanism of stimulation of neoplastic growth in ovaries,[13] pituitary,[14] thymus,[15] and thyroid[16] after radiation under certain conditions has also been demonstrated. We believe it likely that the stimulation to early onset of other neoplasms invoked by total-body irradiation may also be partially mediated by indirect mechanisms.

It is interesting to consider the work of McCay[17,18] related to problems of aging. In these studies, prolongation of life span of rats above that considered "normal" for the controls was achieved by caloric restric-

tion with growth inhibition in the presence of a diet adequate in known essential nutritious constituents. The onset of neoplasms was delayed in these retardation studies. Since longevity, growth suppression, and appearance time of tumors have been altered by changes of nutrition alone, a study of the nutrition of postirradiated animals seems indicated. Our animals have all received a standard laboratory diet fed ad libitum. We have, as yet, no data bearing on the relative consumption of food in the two groups or their relative efficiency of utilization of ingested food. Such studies are planned for the near future.

Cataracts appear in the irradiated animals coincident with the mean weight decline experienced during the 7- to 12-month postirradiation period, suggesting that weight loss may be related to blindness and consequent failure to eat. Since rats are primarily nocturnal animals used to foraging in the dark, this explanation has not appealed to us. As a check against this possibility, however, the weight curve of another group of postirradiated animals protected from cataracts by head shielding was compared with our totally irradiated animals. A similar weight decline during the 7- to 12-month postirradiation period was observed in these rats with unimpaired vision.

It has been suggested that selection through acute postirradiation mortality yields a group of survivors whose relative radioresistant state is associated with characteristics that, in part at least, explain the various features of the late-radiation-effects syndrome. It should be emphasized here that the 1000 r hypoxic radiation used represents a 30-day L. D_{9}. Ninety-one per cent of the animals survived. Selection here is limited and is not adequate to explain the shortened life span, the accelerated onset of neoplasia, or the high incidence of nephrosclerosis.

Skin ulcers, epilation, and cataracts are well known as delayed complications of direct and local radiation injury. Their presence in the totally irradiated animal might logically be interpreted as further evidence of direct radiation injury. The unexpected high incidence of these diseases in our older control animals raises the alternative probability that epilation, skin ulceration, and cataracts following many months after total body irradiation are, in part, further manifestations of accelerated senility, rather than entirely delayed reactions to the ionizations within the tissues themselves.

Summary

Eighty-three female Wistar rats surviving a single exposure to 1000 r hypoxic total-body irradiation at 4 months of age and eighty-four controls of the same stock were observed throughout their life span.

The irradiated animals were all dead two years after irradiation, with a mean survival of 15 months. This represents a 39% shortening of postirradiation life span, compared to the 24½-month mean survival of the controls. This reduction of life span following hypoxic irradiation is comparable to that reported in rodents following total-body irradiation in air, if the dose is expressed in each case as a per cent of the L. D_{50} dose.

Irradiated rats were also retarded in growth, weighing approximately 18% less than the controls 20 months post irradiation.

All irradiated rats surviving at least 9½ months developed cataracts, with a mean appearance time of 10½ months. Aged control rats also acquired cataracts, appearing first at 26½ months.

Eighteen irradiated rats developed severe bilateral nephrosclerosis, a disease not observed in the controls.

Large pituitary adenomas were common in the controls but absent in the irradiated group.

Other major pathologic findings in the irradiated rat were closely similar to those found in control rats of advanced age.

Tumors, as a whole, appeared sooner in the irradiated rats but not in greater final numbers. The incidence of tumors in both irradiated and control series generally followed the pattern characteristic of the ani-

mal strain, with reproductive and endocrine loci predominating.

Ovarian tumors appeared to be specifically increased by the irradiation exposure.

Lymphomas were not increased or accelerated by radiation under these conditions in this low-incidence strain.

In general, the early onset of neoplasia in the irradiated rat seems best explained as one aspect of an accelerated aging process. Other diseases prevalent in aged rats, such as cataracts, acute inflammations, epilation, and skin ulcerations, were similarly accelerated to a comparable degree.

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REFERENCES

1. Furth, J., and Furth, O. B.: Neoplastic Disease Produced in Mice by General Irradiation with X-Rays: I. Incidence and Types of Neoplasms, Am. J. Cancer 28:54, 1936.

2. Henshaw, P. S.: Leukemia in Mice Following Exposure to X-Rays, Radiology 43:279, 1944.

3. Lamson, B. G.; Meek, R. A., and Bennett, L. R.: Late Effects of Total-Body Roentgen Irradiation: II. The Influence of Fractionated and Single Radiation Doses on the Incidence of Tumors, Nephrosclerosis, and Adrenal Vacuolation in Wistar Rats During Various Periods of Post-irradiation Survival, A. M. A. Arch. Path. 64: 505, 1957.

4. Lamson, B. G.; Ewell, L. H., and Bennett, L. R.: Neoplasms in Female Wistar Rats Occurring Spontaneously and Following 1000 r Anoxic Total Body Irradiation, Fed. Proc. 15:521, 1956.

5. Lamson, B. G.; Billings, M. S.; Ewell, L. H., and Bennett, L. R.: Late Effects of Total-Body Roentgen Irradiation: IV. Hypertension and Nephrosclerosis in Female Wistar Rats Surviving 1000 r Hypoxic Total-Body Irradiation, A. M. A. Arch. Path., this issue, p. ???.

6. Dowdy, A. H.; Bennett, L. R., and Chastain, S. M.: Protective Action of Anoxic Anoxia Against Total Body Roentgen Irradiation of Mammals, Radiology 55:879, 1950.

7. Friedman, M., and Freed, S. C.: Microphonic Manometer for Indirect Determination of Systolic Blood Pressure in the Rat, Proc. Soc. Exper. Biol. & Med. 70:670, 1949.

8. Bennett, L. R.; Chastain, S. M.; Flint, J. S.; Hansen, R. A., and Lewis, A. E.: Late Effects of Roentgen Irradiation: I. Studies on Rats Irradiated Under Anoxic Anoxia, Radiology 61:411, 1953.

9. Blair, H. A.: Data Pertaining to Shortening of Life Span by Ionizing Radiation, University of Rochester Project, Atomic Energy Project Report UR-442, 1956.

10. Furth, J.; Upton, A. C.; Christenberry, K. W.; Benedict, W. H., and Moshman, J.: Some Late Effects in Mice of Ionizing Radiation from an Experimental Nuclear Detonation, Radiology 63:562, 1954.

11. Blair, H. A.: A Formulation of the Injury, Life Span, Dose Relations for Ionizing Radiations, University of Rochester Project, Atomic Energy Project Report UR-206, 1952.

12. Blair, H. A., in Report of the Committee on Pathologic Effects of Atomic Radiation, National Research Council, Publication 452, Appendix IV, 1956.

13. Lick, L.; Kirschbaum, A., and Mixer, H.: Mechanism of Induction of Ovarian Tumors by X-Rays, Cancer Res. 9:532, 1949.

14. Furth, J., and Barnett, W. T., Jr.: Hormone-Secreting Transplantable Neoplasms of the Pituitary Induced by I^{131}, Proc. Soc. Exper. Biol. & Med. 78:222, 1951.

15. Kaplan, H. S.: Preliminary Studies of the Effectiveness of Local Irradiation in the Induction of Lymphoid Tumors in Mice, J. Nat. Cancer Inst. 10:267, 1949.

16. Doniach, I.: The Effect of Radioactive Iodine Alone and in Combination with Methylthiouracil upon Tumour Production in the Rat's Thyroid Gland, Brit. J. Cancer 7:181, 1953.

17. McCay, C. M.; Maynard, L. A.; Sperling, G., and Barnes, L. L.: Retarded Growth, Life Span, Ultimate Body Size, and Age Changes in the Albino Rat After Feeding Diets Restricted in Calories, J. Nutrition 18:1, 1939.

18. McCay, C. M.: in Cowdry's Problems of Ageing: Biological and Medical Aspects, edited by A. J. Lansing, Ed. 3, Baltimore, Williams & Wilkins Company, 1952.

Late Effects of Total-Body Roentgen Irradiation

IV. Hypertension and Nephrosclerosis in Female Wistar Rats Surviving 1000 r Hypoxic Total-Body Irradiation

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The kidney is one of the more radioresistant organs when damage from ionizing irradiation is appraised by evidence of acute histologic injury. Large doses of radiation of the magnitude used in radiation therapy of neoplasms can, nevertheless, produce acute and delayed renal injury, as shown experimentally in dogs[1,2] and in human cases of "radiation nephritis" where the kidney has been included within the therapeutic radiation field.[3,4] When smaller doses in the range of 500 to 1000 r have been delivered to the total body or to a portion of the body which includes the kidney, delayed renal injury and hypertension in rats[5-9] and delayed renal injury in mice[10,11] have also been described.

After the 1000 r hypoxic total-body radiation of this study a severe characteristic nephrosclerosis was observed in 46% of the autopsied rats. A high prevalence of hypertension has also been observed in those postirradiated rats of this study that have remained free of this renal disease. Nephrosclerosis of the type under consideration here, on the other hand, was not present in those total-body-irradiated rats with a history of normal blood pressure. Recent observations in this laboratory indicate that nonirradiated hypertensive rats can also develop a nephrosclerosis similar, if not identical, to the renal disease of the "late effects" syndrome of total body irradiation. The possible influence of postirradiation hypertension in the evolution of the nephrosclerosis of this "late effects" syndrome is the principal subject of this report. Our experience with the variable prevalence of postirradiation nephrosclerosis in this laboratory over a period of nine years is also included.

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Methods

Sixty-one female Wistar rats surviving 1000 r total-body irradiation delivered during a hypoxic state of 5% oxygen tension and 64 control rats of the same genetic background, size, age, and sex were observed for the duration of their natural life span under the conditions in our laboratory. Techniques of irradiation and subsequent selection of these 125 rats are detailed in a preceding paper.[12] Irradiated and control rats were approximately 4 months of age at the time of radiation exposure. Subsequent charts measure time in months from this arbitrary starting date.

The rats were housed four to a cage in a room isolated from other animals for the next 15 months. Their Rockland Rat Diet contained 0.88% Na^+, 1.12% K^+, and 21.0% crude protein. During the early months of the postirradiation period, one-half of both the irradiated and control groups received a prolonged systematic course of prophylactic antibodies. For details, refer to the preceding publication.[12] This antibiotic regimen introduced no apparent effect upon the incidence of either hypertension or nephrosclerosis.

Animal quarters were not air conditioned during the first 15 months of this study, and daily fluctuations of temperature of 10 degrees (F) undoubtedly occurred. During the winter months mean temperatures were, no doubt, consistently below that considered optimal for ideal rat hygiene. After 15 months the colony was moved to a modern air-conditioned vivarium.

Sytolic blood pressure was measured by the tail-cuff method of Friedman and Freed,[18] with use of light Myotal * anesthesia, 0.1 cc. per animal intraperitoneally. Blood pressures were recorded at approximate monthly intervals, commencing with the 13th month after irradiation. The entire colony population was measured in rotation without selection, but in the early months of the blood pressure study the larger number of surviving animals required longer than a month for these observations. Listed readings are the average of the several measurements which were made if any uncertainty existed as to the acoustic end-point of the measuring technique. Animals were observed until death occurred or was imminent from natural causes. Complete autopsies were performed in all cases.

Severity of all chronic renal disease observed histologically is graded 1 through 4. The nephrosclerosis associated with total-body radiation exposure is recognized histologically by a characteristic generalized bilateral thickening of glomerular tufts and arterioles with a periodic acid-Schiff (PAS)-positive material and many cast-filled dilated tubules, without inflammatory-cell influx, as detailed in a previous publication.[8] In kidneys with minimal changes graded as 1+, early stages of nephrosclerosis of this type associated with total-body irradiation cannot always be distinguished with certainty from the focal nephrosclerotic scars so common in older rats. On the other hand, nephrosclerosis associated with total-body irradiation graded 2+ or more is a highly characteristic disease, with a 4+ lesion converting the entire kidney to a scarred and sieve-like state which is so severe that it may be readily recognized by macroscopic examination of the tissue slide. To facilitate distinction between the characteristic renal lesion of the total-body-irradiated rat and the minor focal renal disease so frequently observed in older animals, kidneys graded as 1+ which include some cases of naturally occurring minimal renal disease have been arbitrarily tabulated in Table 3 as nephrosclerosis absent. The unqualified term, nephrosclerosis, as used henceforth in this paper, refers to the renal change associated with total-body irradiation that is characteristic in appearance and more extensive than the minimal renal lesions so frequently observed in this species.[8] Data illustrating the variable prevalence of this nephrosclerosis as defined above in other groups of female Wistar rats after 1000 r hypoxic irradiation in this laboratory under closely similar conditions are included. These studies extend over a nine-year period, with the initial group preceding[5,8] and the last four experiments following the detailed study reported here.

* Stated to consist of pentobarbitol sodium with mephenesin.

TABLE 1.—*Mean Systolic Blood Pressure at Monthly Intervals in Normal and Irradiated Female Wistar Rats Surviving 1000 r Hypoxic Total-Body Irradiation*

Mo. After Beginning Experiment	Total-Body Irradiation 1000 r			Nonirradiated Controls		
	Mean, Mm.	σ	No.	Mean, Mm.	σ	No.
13	159	±32	55	109	±15	27
14	170	±44	27	121	±21	40
15	159	±34	35	114	±17	59
16	159	±20	31	120	±15	59
17	171	±27	20	122	±11	5
18	184	±45	18	116	±14	54
19	166	±37	15	125	±19	52
20	130	±37	10	123	±20	49
21	149	±40	6	127	±20	49
22	147	±33	6	136	±29	4
23	175	..	2	122	±16	34
24				130	±20	4
25				119	±19	32
26				125	..	2
27				..	..	..
28				122	±19	23
29				127	±26	17

Results

Prevalence of Hypertension.—At the beginning of the 13th month 59 irradiated rats and 63 controls were still alive. Mean blood pressures of those irradiated and control rats measured during each month are recorded as millimeters of mercury in Table 1. Standard deviations of the mean values are shown where four or more values contribute to the mean.

Mean systolic pressures are already substantially elevated in the irradiated animals by 13 months post irradiation, when readings were first obtained. This mean elevation generally persisted for the duration of the life span of the irradiated colony, with the exception of the mean value at 20 months post irradiation, when there was little elevation above the controls. This low blood pressure mean at 20 months was obtained at a time when the mean body weight of the irradiated group had declined approximately 10% and the animals appeared to be clinically suffering from epidemic respiratory disease. This difficulty was largely resolved one month later, when the animals appeared in improved condition. Blood pressures were again elevated at this later time.

TABLE 2.—*Prevalence of Nephrosclerosis of All Types in Irradiated and Control Female Wistar Rats Surviving 1000 r Hypoxic Total-Body Irradiation*

Severity of Nephrosclerosis (Graded 1-4)	Survival After Beginning Experiment, Mo.*							
	2-12		13-18		19-24		25-30	30-33
	Ir	C	Ir	C	Ir	C	C	C
4+	1		3		1			
3+			10		2		1	
2+			8		2			
1+			7		5	2	2	1
0	1	1	13	6	6	12	19	19
N. A.†			2	1				
Total deaths	2	1	43	7	16	14	22	20

* Ir indicates irradiated; C, nonirradiated control.
† Microscopic evaluation not available.

Prevalence of Nephrosclerosis.—The prevalence of nephrosclerosis of all types and degrees in 123 of these same rats during several periods of survival is shown in Table 2. With one exception, all chronic renal disease graded 2+ or more occurred in the irradiated group and was of the type characteristic of total-body radiation exposure. The increased frequency of 1+ lesions in the irradiated group undoubtedly represents the summation of naturally occurring disease and examples of minimal radiation associated nephrosclerosis.

The majority of deaths in the irradiated group occurred in the interval 13 to 18 months post irradiation. Approximately half of these had nephrosclerosis graded at least 2+ at autopsy. Kidneys from two irradiated rats dying during this period were not available for histologic study. The longer term survivors living 19-24 months post irradiation showed a similar, although certainly no larger, incidence of the disease. An over-all incidence of 46% with nephrosclerosis graded 2+ or more is observed based upon the 59 rats whose kidneys were available for microscopic study.

In the control animals only one rat dying during the 25- to 30-month survival period had bilateral generalized renal disease of a degree comparable to the more severely injured kidneys of the irradiated group. The kidneys in this case did show glomerular basement membrane thickening and dilated cast-filled tubules, but, in addition, the parenchyma was infiltrated by many plasma cells and lymphocytes, suggesting chronic pyelonephritis. Generalized renal disease resembling the nephrosclerosis of the irradiated animals was not found in any other case, even though roughly one-third of the control animals survived for longer than 30 months after the onset of the study, reaching an actual age of 34 months at this time. Scattered examples of naturally occurring renal disease of minor extent graded 1+ were, however, observed in control rats living more than 18 months after onset of the study.

Relationship of Nephrosclerosis to Maximum Observed Blood Pressure.—Blood pressure readings and histologic evaluation of the kidneys are both available in 54 of the 59 rats surviving at least 13 months post irradiation. The mean values of maximal blood pressure determinations from each of these 54 irradiated rats and from the 63 control rats are shown at the bottom of Table 3. The irradiated group is subdivided into those with and without nephrosclerosis (2+ or more). Mean maximal blood pressure of the total 54 irradiated rats is 193 mm. ±35 mm. Hg, compared with 139 mm. ±19 mm. for the controls. In the irradiated group hypertension is not limited, however, to those rats with nephrosclerosis. The mean maximal blood pressure of irradiated rats without nephrosclerosis is almost as high, 185 mm. ±35 mm., as in those with nephrosclerosis, 202 mm. ±31 mm. This observation is also supported by the monthly mean pressures. When the blood pressures from irradiated animals with and without nephrosclerosis are separately tabulated, both groups are again found to have, in general, much higher monthly pressures than the controls. The elevated pressures of those rats without nephrosclerosis reach or exceed the levels of those with nephrosclerosis only at one point 19 months post irradiation.

TABLE 3.—*Magnitude of Maximal Systolic Blood Pressures in Control and Irradiated Rats With and Without Nephrosclerosis*

Maximum Blood Pressure, Mm Hg	Control (Nephrosclerosis Absent)	Irradiated	
		Nephrosclerosis Present	Nephrosclerosis Absent
200 and above	0	13	11
180–199	0	7	4
160–179	11	3	6
140–159	22	1 (2+)*	6
120–139	18	1 (4+)*	1
<120	12		1
Rats, No.	63	25	29
Mean maximum pressure	139 mm. ±19 mm.	202 mm. ±31 mm.	185 mm. ±35 mm.
		193 mm. ±35 mm.	

* Indicates severity of nephrosclerosis in this rat.

The magnitude of maximal systolic blood pressures in rats with and without nephrosclerosis is also shown in Table 3. Eleven of the control rats had one or more maximal readings between 160 and 179 mm., and twenty-two had readings at some time between 140 and 159 mm. Hg. Many of these higher readings were in very old animals. No control values above 180 were observed at any time. The frequency of high maximal readings above 200 mm. Hg among the irradiated groups is shown. These mean maximal pressures are slightly higher in those irradiated rats with nephrosclerosis. The high pressures also found in the irradiated animals with kidneys free of nephrosclerosis, however, mitigates against this disease being a necessary prerequisite for the postradiation hypertensive state. In fact, we have selectively killed several post-irradiated animals not included in this series when blood pressures of over 200 mm. have first been discovered. Histologically *normal* kidneys were found.

On the other hand, almost all the irradiated animals with nephrosclerosis had hypertension. The one exception is indicated in Table 3, when a maximal systolic pressure of 130 mm. was associated with severe (4+) nephrosclerosis. This particular animal had its blood pressure measured only once, 13 months post irradiation. It appeared in terminal condition at this time and was then killed. Autopsy revealed acute purulent meningitis and pneumonia. Hypertension could have existed prior to this terminal illness. The one irradiated animal with nephrosclerosis and a maximal blood pressure in the 140-159 range had highest readings of 150, 156, and 158 mm. on different occasions. Nephrosclerosis here was moderate (2+), or the least degree of involvement that can be distinguished with certainty from naturally occurring minimal renal disease.

Duration of Hypertension and Incidence of Nephrosclerosis in Irradiated Rats.—Monthly blood pressure readings have been further studied according to the number of months preceding death of the irradiated animal at the time the pressure was recorded. These blood pressures so arranged were when compared in irradiated rats with and without nephrosclerosis. No significant difference in the duration of hypertension before death, as measured by these monthly readings, was observed in the two groups. Several rats had blood pressure readings as high as 180 mm. Hg on at least one occasion four or five months before death, yet nephrosclerosis was not found at autopsy. Other animals with well-developed nephrosclerosis had blood pressures of 180 mm. or above only at readings taken one or two months prior to death.

Adrenal, Kidney, and Heart Weights in Irradiated and Control Rats.—Tables 4 and 5 are compilations of adrenal, kidney, and

TABLE 4.—*Adrenal, Kidney, and Heart Weights in Nonirradiated Control Female Wistar Rats Living for Various Periods After Onset of Radiation Study* *

	Survival After Onset of Radiation Study, Mo.											
	13–18			19–24			25–30			31–33		
	Mean	σ	No.	Mean	σ	No.	Mean	σ	No.	Mean	σ	No.
Adrenal, gm.	0.078	±0.042	19	0.085	±0.023	16	0.118	±0.068	17	0.120	±0.042	17
Adrenal, gm/kg.	0.343	±0.183	13	0.325	±0.153	17	0.445	±0.205	17	0.489	±0.161	16
Kidney, gm.	2.12	±0.43	7	2.19	±0.32	16	2.29	±0.40	17	2.52	±0.44	17
Kidney, gm/kg.	8.97	±0.70	7	8.55	±1.55	16	8.69	±3.20	17	10.0	±2.50	17
Heart, gm.	0.934	±0.235	7	0.999	±0.123	14	0.943	±0.156	18	1.09	±0.113	17
Heart, gm/kg.	3.49	±0.574	7	3.60	±0.535	14	3.77	±1.12	18	4.36	±1.08	17

* Rats were irradiated at approximately 4 months of age.

heart weights during various periods of survival of the control and irradiated animals, respectively. In the controls in Table 4 an increase in adrenal size is shown in animals surviving over 24 months. The actual age of these rats is four months greater than the time intervals listed. Kidneys and hearts of the oldest group of controls also appear to be slightly enlarged. Table 5 lists weights of the same organs in irradiated rats dying during the second 12 months of the 24-month postirradiation survival period. Adrenal glands are not significantly larger than the controls on either an actual weight or proportion of body weight basis. Mean kidney weights of the irradiated animals also do not significantly differ from the controls, even though nephrosclerosis is prevalent in these irradiated animals. The nephrosclerotic kidneys are obviously not small contracted organs. This is further shown in the comparison between those irradiated animals with and without nephrosclerosis. No significant difference in actual kidney size is evident. Hearts of the irradiated rats are generally larger than the controls when expressed as a proportion of total body weight. This observation is not restricted to those rats with nephrosclerosis, lending support to the concept that hypertension is not restricted to those rats with demonstrable renal disease.

The prevalence of nephrosclerosis of the type associated with total-body radiation exposure in six consecutive studies spanning a nine-year period are tabulated in Table 6. These experiments have all been performed on female Wistar rats obtained from the same supplier and irradiated with 1000 r during 5% oxygen inhalation at age 3 to 4 months. The group of rats indicated had their heads shielded from the irradiation exposure. All others received total-body irradiation. Some variation in diet composition and external environment has occurred over this period, but all were fed ad libitum with commercial rat diets and housed four to a cage. The total prevalence of nephrosclerosis during the 7- to

TABLE 5.—*Adrenal, Kidney, and Heart Weights in Female Wistar Rats Surviving 1000 r Hypoxic Total-Body Radiation for Longer than Twelve Months* *

	Postirradiation Survival, Mo.					
	13–18			19–24		
Organ	Mean	σ	No.	Mean	σ	No.
Adrenal, gm.	0.078	±0.028	43	0.079	±0.019	18
Adrenal gm/kg. total body weight	0.380	±0.168	34	0.370	±0.134	18
Kidney, gm.	2.02	±0.54	42	2.02	±0.29	16
With nephrosclerosis	2.11	±0.45	20	1.96	±0.237	5
Without nephrosclerosis	1.84	±0.35	20	1.99	±0.330	10
Kidney, gm/kg. total body weight	9.40	±2.10	33	9.02	±1.50	16
Heart, gm.	0.893	±0.148	36	0.920	±0.115	14
With nephrosclerosis	0.978	±0.084	19	0.920	±0.103	5
Without nephrosclerosis	0.798	±0.161	17	0.920	±0.120	9
Heart, gm/kg. total body weight	4.28	±0.826	30	4.25	±0.516	14
With nephrosclerosis	4.49	±0.767	15	4.55	±0.513	5
Without nephrosclerosis	4.62	±1.06	15	4.09	±0.438	9

* Wet weights.

TABLE 6.—*Prevalence of Nephrosclerosis in Six Consecutive Groups of Female Wistar Rats After 1000 r Total- or Near-Total-Body Irradiation During Hypoxia* **

Year Study Commenced	Rats, No.	Postirradiation Survival, Mo. 7-12	13-18	19-24
1949 *	21	8/9	4/9	1/3
1953 †	58	1/1	21/41	5/16
1954	65	0/19	2/22	4/24
1955	17	2/6	4/6	0/5
1955 ‡	22	1/3	8/12	5/7
1955	21	0/9	1/11	1/1

* Fractions indicate the proportion of rats with nephrosclerosis among deaths during each survival period.

† Present data.

‡ Head shielded.

24-month survival periods tabulated ranges from 9% to 64%, with the present study occupying an intermediate position. Prevalence of hypertension has run parallel to the frequency of renal disease.

Comment

Immediate and delayed renal damage after direct x-irradiation of the kidneys with doses in the 2000-3000 r range delivered without the protection of anoxia is well documented.[1-4] When delayed nephrosclerosis was later observed as one of the late sequelae of total-body irradiation, a similar direct radiation effect on the kidney might be assumed notwithstanding the absence of signs of early damage and the substantially smaller doses of 500 to 1000 r to the kidneys involved in the total-body exposure. In our own work, with use of 1000 r delivered while the rats are subjected to a hypoxic environment of 5% oxygen and 95% nitrogen, the total-body radiation exposure represents approximately an L. $D._9$ in 30 days.[12] If renal damage from ionizing radiation is directly proportional to the per cent of a lethal total body dose administered (a relationship that is not yet firmly established), then 1000 r of hypoxic total body radiation should be comparable in its renal effects to a total-body dose without anoxia also lethal to 9% in 30 days, or approximately 425-500 r. To the best of our knowledge, permanent renal injury has not been observed following direct radiation limited to the rat kidney with doses of this relatively small size. The recent publication by Maisin et al.[9] in which unilateral renal disease was observed in rats following 850 r to a radiation field that included one kidney indicates, however, that our 1000 hypoxic r may not be far below the threshold for demonstrable direct-radiation injury to the kidney.

Extension of our data to reveal the occurrence of hypertension in some of the postirradiated rats without nephrosclerosis, as well as in those with this disease, raises the possibility that this postirradiation hypertension may precede the evolution of histologically demonstrable renal disease following radiation at this dose level.

Experimental hypertension in the nonirradiated rat has been associated with renal lesions bearing some similarity to the disease under consideration here. For example, Wood and Ethridge,[14] in 1933, described similar glomerular changes in the renal remnant of Wistar rats made hypertensive by surgical removal of a large portion of the total renal mass. Loomis,[15] using unilateral nephrectomy and partial infarction of the surviving kidney to reduce renal substance to 25% of the original total, also demonstrated in the residual noninfarcted renal tissue vascular and tubular changes similar to those observed in our rats. Ninety per cent of her animals had hypertension above 160 mm. Hg. Two-kidney experiments in rats,[16-19] where one kidney has been left intact and the other subjected to some form of unilateral renal manipulation, have provided opportunities to study the effect of renal hypertension upon a nonmanipulated presumably normal kidney. The histologic descriptions and photomicrographs of Wilson and Byrom[16] depicting the chronic changes in their rats in this circumstance are particularly similar to the lesion under consideration here. Our rats have not shown the acute fibrinoid necrosis of arterioles and intimal thickening of larger kidney arteries also described by these

authors. Friedman and his associates,[17] on the other hand, in similar two-kidney experiments where hypertension was of less duration, mention only infrequent striking histologic changes. The very great similarity between the nephrosclerosis observed in hypertensive irradiated rats and previously described renal lesions in nonirradiated rats with known hypertension is suggestive.

The cause of the postirradiation hypertension in these totally irradiated rats cannot be determined from this study. Most examples of renal hypertension associated with directly induced radiation nephritis, as described in Grossman's[4] extensive review, involved radiation doses sufficiently large to produce initially some evidence of acute renal injury. Histologic evidence of such immediate renal damage in the first few weeks and months after radiation exposure is absent after hypoxic 1000 r total-body exposure. Obviously, renal injury at the submicroscopic level may still have occurred. Our efforts to detect evidence of impaired renal function during this latent period when the kidneys are histologically normal in appearance will be described in a later report.

In our own laboratory more recently completed experiments with this same strain of Wistar rats involving radiation of the entire body or the majority of the body with 1000 r (hypoxic) have produced a high incidence of nephrosclerosis in only two of four studies. This variable incidence of nephrosclerosis in the irradiated animal may represent only biologic variation in the Wistar rat strain in use over a nine-year period. We are also exploring the possibility that as yet poorly defined variations in the postirradiation environment of the rat colony may explain this observation. In our still inconclusive efforts to define any specific environmental conditions conducive to maximum incidence of post-total-body-radiation hypertension, we have produced hypertension in some of our nonirradiated control rats as well. It is of interest that several of these control animals made hypertensive by unilateral nephrectomy and a chronic 1% sodium chloride solution in lieu of tap water have also developed nephrosclerosis indistinguishable by ordinary histologic techniques from the nephrosclerosis of total-body irradiation observed in this laboratory. This observation in nonirradiated animals, to be subsequently described in detail, lends support to the concept that post-total-body-radiation nephrosclerosis in the Wistar rat is not a unique radiation-induced lesion but rather a sequela of radiation-induced hypertension. The role that renal irradiation may play in the genesis of this hypertensive state must await further study, which should include the effects of irradiation at this same dose level when restricted to the kidney.

Summary

Systolic blood pressure levels were studied in 59 female Wistar rats surviving 1000 r hypoxic total-body irradiation and in 63 controls of the same age.

Mean blood pressure of the irradiated rats at monthly intervals from 13 to 23 months post irradiation averaged 39 mm. Hg greater than the controls of the same age.

Forty-six per cent of the irradiated rats also developed bilateral nephrosclerosis.

The mean value of maximal blood pressure determinations from individual rats was 193 mm. Hg in the irradiated group, compared to 139 mm. Hg in the controls. Mean blood pressure maxima in the irradiated rats without severe renal disease was almost as high (185 mm.) as in those with nephrosclerosis (202 mm.).

No correlation between duration of hypertension and eventual development of nephrosclerosis could be made, but rats with nephrosclerosis in no case had normotensive histories.

Direct renal irradiation may be necessary to initiate the hypertensive phase of the total-body radiation "late effects" syndrome, but inclusion of the kidneys within such radiation field does not consistently produce

nephrosclerosis at the 1000 r (hypoxic) dose level.

There are unreported observations that this postirradiation nephrosclerosis of total-body exposure does not differ in histologic appearance from the renal lesion observed in nonirradiated rats made hypertensive by unilateral nephrectomy and a 1% salt drinking solution.

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REFERENCES

1. Hartman, E. W.; Bolliger, A., and Doub, H. P.: Experimental Nephritis Produced by Irradiation, Am. J. M. Sc. 172:487, 1926.

2. Page, I. H.: Production of Nephritis in Dogs by Roentgen Rays, Am. J. M. Sc. 191:251, 1936.

3. Kunkler, P. B.; Farr, R. F., and Luxton, R. W.: The Limit of Renal Tolerance to X-Rays: Investigation into Renal Damage Occurring Following Treatment of Tumors of Testis by Abdominal Baths, Brit. J. Radiol. 25:190, 1952.

4. Grossman, B. J.: Radiation Nephritis, J. Pediat. 47:424, 1955.

5. Bennett, L. R.; Chastain, S. M.; Flint, J. S.; Hansen, R. A., and Lewis, A. E.: Late Effects of Roentgen Irradiation: I. Studies on Rats Irradiated Under Anoxic Anoxia, Radiology 61:411, 1953.

6. Lamson, B. G.; Bennett, L. R., and Billings, M.: Hypertension and Nephrosclerosis in Wistar Rats Following Total Body Irradiation Under Anoxia Conditions, Fed. Proc. 14:425, 1955.

7. Billings, M. S.; Bennett, L. R., and Lamson, B. G.: Hypertension and Renal Disease in Wistar Rats Following 1000 r Anoxic Total Body Irradiation, Fed. Proc. 15:508, 1956.

8. Lamson, B. G., Meek, R. A., and Bennett, L. R.: Late Effects of Total-Body Roentgen Irradiation: II. The Influence of Fractionated and Single Radiation Doses on the Incidence of Tumors, Nephrosclerosis, and Adrenal Vacuolation in Wistar Rats During Various Periods of Postirradiation Survival, A. M. A. Arch. Path. 64:505, 1957.

9. Maisin, J.; Maldague, P.; Dunjic, A., and Maisin, H.: Syndromes mortels et effets tardifs des irradiations totales et subtotales chez le rat, J. belge radiol. 40:346, 1957.

10. Furth, J.; Upton, A. C.; Christenberry, K. W.; Benedict, W. H., and Moshman, J.: Some Late Effects in Mice of Ionizing Radiation from an Experimental Nuclear Detonation, Radiology 63:562, 1954.

11. Cole, L. J.; Nowell, P. C., and Ellis, M. E.: Incidence of Neoplasms and Other Late Lesions in Mice Protected Against Lethal X-Ray Doses by Spleen Homogenate, J. Nat. Cancer Inst. 17:435, 1956.

12. Lamson, B. G.; Billings, M. S.; Meek, R. A., and Bennett, L. R.: Late Effects of Total-Body Roentgen Irradiation: III. Early Appearance of Neoplasms and Life-Shortening in Female Wistar Rats Surviving 1000 r Hypoxic Total-Body Irradiation, A. M. A. Arch. Path., this issue, p. 311.

13. Friedman, M., and Freed, S. C.: Microphonic Manometer for Indirect Determination of Systolic Blood Pressure in the Rat, Proc. Soc. Exper. Biol. & Med. 70:670, 1949.

14. Wood, J. E., Jr., and Ethridge, C.: Hypertension with Arteriolar and Glomerular Changes in the Albino Rat Following Subtotal Nephrectomy, Proc. Soc. Exper. Biol. & Med. 30:1039, 1933.

15. Loomis, D.: Hypertension and Necrotizing Arteritis in the Rat Following Renal Infarction, Arch. Path. 41:231, 1946.

16. Wilson, C., and Byrom, F. B.: The Vicious Circle in Chronic Bright's Disease: Experimental Evidence from the Hypertensive Rat, Quart. J. Med. 34:65, 1941.

17. Friedman, B.; Jarman, J., and Klemperer, P.: Sustained Hypertension Following Experimental Unilateral Renal Injuries: Effect of Nephrectomy, Am. J. M. Sc. 202:20, 1941.

18. Schroeder, H. A., and Neuman, C.: Arterial Hypertension in Rats: II. Effects on the Kidneys, J. Exper. Med. 75:527, 1942.

19. Halpert, B., and Grollman, A.: Structural Changes in the Kidneys of Rats with Experimental Chronic Hypertension, Arch. Path. 43:559, 1947.

20. Selye, H.; Hall, C. E., and Rowley, E. M.: Malignant Hypertension Produced by Treatment with Desoxycorticosterone Acetate and Sodium Chloride, Canad. M. A. J. 49:88, 1943.

21. Selye, H., and Stone, H.: Pathogenesis of the Cardiovascular and Renal Changes Which Usually Accompany Malignant Hypertension, J. Urol. 56:399, 1946.

22. Masson, G. M. C.; Hazard, J. B.; Corcoran, A. C., and Page, I. H.: Experimental Vascular Disease Due to Desoxycorticosterone and Anterior Pituitary Factors: Comparison of Pathologic Changes, Arch. Path. 49:641, 1950.

23. Friedman, S. M.; Friedman, C. L., and Nakashima, M.: Sustained Hypertension Following the Administration of Desoxycorticosterone Acetate (Adrenocortical Preparation), J. Exper. Med. 93:361, 1951.

The Validity of Tissue Mast-Cell Counts in Postmortem Material

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Two reports have appeared in the recent literature concerning the effects of time and temperature on the stability of certain substances in human and animal tissues obtained post mortem.[1,2] Information of this type is important in human pathology, since, too often, an appreciable interval elapses between the death of the patient and the time of histological fixation or chemical examination of the tissues. The list of substances studied by these two groups of workers does not include the tissue mast cells—structures which are of particular interest to us since we have been engaged for some time in their numerical estimation in the myocardium and subsequent comparison with the severity of coronary atherosclerosis. The period between death and autopsy in our series has been so variable that we realized our results would be unacceptable unless it were shown that time and temperature had no significant effect on the number of these cells in unfixed tissues. The present report deals with the number of mast cells in human and rat tissues with various fixatives and varying intervals of time after removal from the body.

Material and Method

The human material was obtained in part from the hearts of five patients on whom autopsy was carried out less than two hours after death. Additional human material consisted of skin and subcutaneous tissue from two surgical amputation cases, the first specimens being fixed within one and one-half minutes of removal from the body. The animal material consisted of segments of myocardium and of skin and underlying tissue from rats, the first blocks of tissue being fixed within a few minutes after killing the animals.

The material from the autopsies on human fatalities was treated as follows. A segment of myocardium was removed from each case and cut into blocks of appropriate size. One block was fixed in 15% formalin immediately (within two hours of death), and the remaining blocks were immersed in isotonic saline and left at room temperature. These latter blocks were removed from the saline and fixed in formalin at from 10 to 14 hours in each of the five cases and at from 20 to 23 hours after death in three cases.

The tissue for the dermal mast cell counts in human surgical material was handled in a different manner. The first blocks of tissue were fixed immediately (within one and one-half minutes after removal) in 15% formalin and in the second case in lead subacetate-formalin as well. The remaining blocks were immersed in saline and left either at room temperature or in the refrigerator at 4.4 C. Representative blocks were fixed appropriately at intervals varying up to 24 hours. The blocks were embedded in paraffin, sectioned at 7μ thickness, and stained with 1:1000 aqueous solution of toluidine blue. The mast-cell counts on these tissues and on the human myocardium mentioned above were made on 100-500 high-power fields and expressed as the number of cells per square centimeter of tissue.

The material from the heart and subcutaneous tissues of rats was fixed in 15% formalin immediately after killing the animal and at 6 and 24 hours after refrigeration at 4 C in a covered jar. Paraffin sections were cut at 7μ and stained with accelerated Giemsa blood stain.[3] Mast-cell counts were made on 300 high-power fields and expressed as the number of cells per square centimeter of tissue.

To check the reliability of formalin as a fixative for mast cells, adjacent blocks of tissue of a spontaneous malignant fibroadenoma of the rat breast were placed in the following fixatives: 15% buffered formalin, absolute alcohol, Bouin's fix-

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ative, formol-alcohol, formol-alcohol with calcium acetate, and a 4% freshly prepared aqueous solution of lead subacetate. The tissue blocks were embedded in paraffin, cut at 7μ, and stained with an accelerated Giemsa blood stain for 4 to 5 minutes; the number of mast cells were counted in 100 consecutive high-power microscopic fields.

The results of these four separate experiments have been analyzed statistically by the "Student" *t* test[4]; *P* values of less than 0.05 were regarded as being significant, those equal to 0.05 were considered to be of borderline significance.

Observations and Comment

Human Myocardium.—The number of mast cells present per square centimeter of human myocardial tissue fixed at different times after death is given in Table 1. It will be seen that there is no statistically significant change with time in this type of material. It is conceded, however, that a change might theoretically have occurred prior to our first observation, which was made approximately two hours after death.

Human Dermis.—The material for this study was obtained within 2 minutes of removal of the tissue from the body, prior to leg amputation, and the effects of time and temperature (and of two different fixatives) were studied up to 24 hours after removal. The mast-cell counts are given in Table 2, and again no consistent statistically significant effect is apparent either from time or temperature. Certain discrepancies from this general result will be noted, particularly when the tissues were fixed in lead subacetate, but these are not consistent.

There appeared to be only one possible flaw in this experiment: Of necessity the skin of the amputated limb of each patient had to be treated preoperatively with disin-

TABLE 1.—*Number of Mast Cells per Square Centimeter of Human Myocardium Fixed at Varying Times After Death*

Case No.	Tissue Specimen	Block, No.	HPF Counted/ Block, No.	Time of Fixation After Death, Hr.	Mean Mast-Cell Count/ Sq. Cm. ± S. E. M.	*P* Values
1	1	3	100	2	240±38.3	>0.10
		3	100	12	187±31.1	
	2	3	100	2	339±65.0	>0.10
		3	100	12	292±45.4	
2	1	3	500	2	233±12.2	>0.10
		3	500	14	218±32.5	
	2	3	500	2	322±17.4	>0.10
		3	500	14	332±23.0	
3	1	3	500	<2	174±14.4	All >0.10
		3	500	10-14	205± 4.0	Except 2 hr.
		3	500	22-23	219± 5.3	Cf. 22-23 hr. >0.05
	2	3	500	<2	271±31.2	
		3	500	10-14	306± 3.3	All >0.10
		3	500	22-23	342±35.3	
4	1	3	300	<2	324±66.4	
		3	300	10-14	287±37.8	All >0.10
		3	300	22-23	327±23.5	
	2	3	300	<2	349±30.9	
		3	300	10-14	357±26.0	All >0.10
		3	300	22-23	366±35.3	
	3	3	300	<2	227±22.2	
		3	300	10-14	229±14.1	All >0.10
		3	300	22-23	190±20.3	
5	1	3	300	<2	285±30.9	
		3	300	10-14	281±33.2	All >0.10
		3	300	22-23	277±34.4	
	2	3	300	<2	267±60.6	
		3	300	10-14	182±42.6	All >0.10
		3	300	22-23	264±24.4	

TABLE 2.—*Number of Mast Cells per Square Centimeter in Human Dermis at Varying Times After Tissue Was Excised from the Body*

Case No.	Time of Fixation After Removal From Body	HPF Counted/ Block, No.	Mean Mast-Cell Count/ Sq. Cm. ± S. E. M. Room Temp.	Refrigerated
1	1.5 min.	200	264±17.5	
	1 hr.	200	237±61.5	219±44.0
(formalin	2.5 hr.	200	281±17.5	176±17.5
fixation)	4.75 hr.	200	211±35.5	158±17.5
	6 hr.	200	316±17.5	132±11.0
	9 hr.	200	176±35.5	184± 9.0
	24 hr.	200	176±17.5	220±11.0
2(a)	1 min.	200	5,264± 596.5	
(formalin	1.25 hr.	200	3,983±1122.5	5,562± 438.5
fixation)	6 hr.	200	4,597± 87.5	4,851± 886.0
	24 hr.	200	3,658± 412.0	3,737± 245.5
2(b)	1 min.	200	5,246± 386.0	
(lead subace-	1.25 hr.	200	3,325± 412.5	6,456±1140.0
tate-formalin	6 hr.	200	5,149± 184.0	3,877± 158.0
fixation)	24 hr.	200	3,307± 132.0	4,983± 736.5

		P Values: Room Temp.	Refrigerated	
Case 1	1.5 min. cf. 6 hr.	>0.10	<0.05	(t=6.38)
	1.5 min. cf. 24 hr.	>0.05	>0.10	
Case 2 Formalin fixation	1 min. cf. 24 hr.	>0.10	>0.10	
Lead subacetate formalin fixation	1 min. cf. 24 hr.	<0.05	>0.10	(t=4.75)

fectants. In Case 1, thimersal (Tincture Merthiolate) was painted on the skin approximately 18 hours before operation, while in Case 2, the leg was washed with hexachlorophene (PhisoHex). We doubt if either of these materials would have any effect on the stability of tissue mast cells because of the results of our experiment with rat tissues described in the next section.

The enormous difference in the numbers of dermal mast cells in these two surgical cases is intriguing. Each had gangrenous toes; in Case 1, the lesion was dry and apparently aseptic, whereas in Case 2 (the one with a very high count) there was obvious sepsis with streaks of lymphangitis in that part of the calf from which the tissue was excised. This chance observation that mast cells may be enormously increased in association with sepsis does not agree with the statement of Janes and McDonald [5] that mast cells are not found in tissues which are the site of acute inflammation.

Rat Myocardium and Dermis.—The results of this phase of the study are shown in Tables 3 and 4. No statistically significant changes are apparent in the myocardial mast-cell counts up to 24 hours after removal of the tissue from the body. The counts in the dermis of the same animals were not so consistently stable, but in general one gains the impression that mast-cell counts in refrigerated tissues of the rat do not change perceptibly up to 24 hours after death. Inasmuch as disinfectants had been applied to the skin of our two human surgical subjects, described above, we have studied the effect of thimersal on the number of dermal mast cells in the skin of a rat. The disinfectant was applied 18 hours before the animal was killed, and counts were made on tissue fixed in formalin at 0, 6, and 24 hours after removal. The mast-cell counts in the thimersal-treated skin were approximately the same as those in control skin.

Finally, it will be observed that formalin fixation was used routinely in this study, although lead subacetate fixation was also

TABLE 3.—*Number of Mast Cells per Square Centimeter of Rat Myocardium at Varying Intervals After Killing*

Case No.	Blocks, No.	HPF Counted/ Block, No.	Time of Fixation After Death, Hr.	Mean Mast-Cell Count/Sq. Cm. ± S. E. M.	*P* Values
1	1	300	0	65±14.0	All values
	1	300	6	75± 9.5	>0.10
	1	300	24	73±12.0	
2	1	300	0	67± 0.9	All values
	1	300	6	54± 8.8	>0.10
	1	300	24	69± 5.8	
3	1	300	0	66± 7.6	All values
	1	300	6	58± 7.3	>0.10
	1	300	24	48± 6.5	

TABLE 4.—*Number of Mast Cells per Square Centimeter of Rat Dermis at Varying Intervals After Killing*

Case No.	Blocks, No.	HPF Counted/ Block, No.	Time of Fixation After Death, Hr.	Mean Mast-Cell Count/Sq. Cm. ± S. E. M.	*P* Value
1	1	300	0	245±54.3	All values
	1	300	6	213± 9.5	>0.10 ex-
	1	300	24	161±14.1	cept 6 hr. cf. 24 hrs. >0.02
2	1	300	0	266±17.9	All values
	1	300	6	219±50.2	>0.10 ex-
	1	300	24	213± 8.2	cept 0 hr. cf. 24 hr. <0.05
3	1	300	0	265±31.7	All values
	1	300	6	287±44.6	>0.10
	1	300	24	204±13.1	
4	1	300	0	196±30.2	All values
	1	300	6	210±23.9	>0.10
	1	300	24	200±32.3	

used for some of the human material. No discrepancies were noted in the human studies, but it was thought worth while to check the mast-cell counts of rat tissues which had been fixed in formalin against the counts in tissues fixed with other materials sometimes reputed to be more satisfactory than formalin. This has been done, with the results given in Table 5. They indicate that, as far as the number of mast cells is concerned, formalin fixation is just as reliable as any of the five other fixatives used. However, we have found that both Bouin's solution and lead subacetate-formalin solution give a more brilliant and clear-cut picture of mast-cell granules than do the other fixatives. Lead subacetate alone gave the poorest picture in this respect.

Summary and Conclusions

Mast-cell counts were made on human and rat tissues fixed at intervals up to 24 hours after removal from the body. Under the conditions of our experiments, no significant changes in the numbers of these cells from time, temperature, or fixation were apparent. It is concluded that mast-cell counts done on refrigerated human tissues which are fixed in formalin within 24 hours of death can be accepted as representing the number of these cells present during life.

TABLE 5.—*Number of Mast Cells per One Hundred High-Power Fields of Parallel Sections of Rat Tumor Tissue Fixed in Various Solutions*

Rat No.	15% Formalin	Absolute Alcohol	Bouin's Fixative	Formol-Alcohol	Calcium Acetate Formol-Alcohol	Lead Subacetate
1	0	0	2	2	23	
2	7	0	3	12	1	
3	0	15	0	0	16	
4	1	1	2	2	0	
5	0	0	11	4	0	
6	2	0	0	1	3	
7	1	25	0	27	4	
8	0	0	0	0	0	
9	9	0	6	0	3	
10	8	1	1	5	0	
11	6	10	15	5	0	
12	0	0	0	16	15	
13	4	0	6	3	0	
14	13	0	18	12	3	
15	11	0	13	0	0	
16	11	0	5	3	0	
17	17	0	14	0	0	13
18	5	0	3	4	9	5
19	1	0	0	0	0	3
20	0	0	1	2	0	1
21	8	2	2	0	0	0
Mean±S. E. M.	5.0±1.1	2.5±1.3	4.9±1.3	4.7±1.5	3.7±1.4	4.4±2.3

P values all >0.10

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REFERENCES

1. Kent, S. P.: Effect of Postmorten Autolysis on Certain Histochemical Reactions, A. M. A. Arch. Path. 64:17, 1957.

2. Smith, D. E.; Robins, E.; Eydt, K. M., and Daesch, G. E.: The Validation of the Quantitative Histochemical Method for Use on Postmortem Material, Lab. Invest. 6:447, 1957.

3. Lillie, R. D.: Histopathologic Technique, New York, The Blakiston Company (division of McGraw-Hill Book Company, Inc.), 1948, p. 222.

4. Fisher, R. A.: Statistical Methods for Research Workers, Edinburgh, Oliver & Boyd, Ltd., 1941.

5. Janes, J., and McDonald, J. R.: Mast Cells: Their Distribution in Various Human Tissues, Arch. Path. 45:622, 1948.

Myocardial Mast-Cell Counts in Coronary Sclerosis

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Following the discovery of the clearing action of heparin on lipemic blood serum, it was suspected by some research workers that this substance might inhibit the deposition of lipid in arterial walls, and thus exert a favorable effect on the progression of atherosclerosis. Since heparin is probably formed in tissue mast cells, still other workers have been intrigued with the possibility that these cells might be concerned in the pathogenesis of the disease. Constantinides in particular has reported evidence in support of this theoretical relationship. He showed, first,[1] that the rabbit, which is highly susceptible to cholesterol-induced atherosclerosis, has a striking deficiency of mast cells in its connective tissues. On the other hand the rat, which is fairly resistant to the cholesterol-induced disease, has connective tissues rich in mast cells.

Constantinides' second report[2] was concerned with the number of mast cells in the myocardium of human subjects with different grades of coronary atherosclerosis. He found a significant decrease in the number of these calls in persons with atherosclerosis compared with the number in persons without atherosclerosis.

We have now attempted to evaluate critically this latter observation. Our technique of counting the mast cells in the myocardium was identical with that used by Constantinides, but our methods of estimating the severity of coronary atherosclerosis were quite different. They were, we believe, more exact. These methods have been described in detail in previous communications[3,4] in which the antemortem levels of the serum lipids in a series of patients under intensive investigation were compared with the severity of atherosclerosis in fatalities in the series.

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Material and Method

The material was obtained from 67 consecutive fatalities among a series of 800 patients who are permanently confined to hospital and on whom serial determinations of the serum lipids are being carried out during life. The patients are mostly psychotics, but the series includes 100 elderly men who are confined to hospital for domiciliary care. The comparison of the antemortem serum lipid levels with the severity of atherosclerosis found at autopsy has been described elsewhere.[3,4] We have now utilized the autopsy material from the same fatalities to determine the relationship of myocardial mast-cell counts to the severity of coronary atherosclerosis.

Our procedure for evaluating the severity of coronary atherosclerosis has also been described in detail,[3,4] and it will be summarized here. Six different indices were used for evaluating the severity of the disease—crude morphological grading, a measurement of the thickness of the largest plaque, and a determination of the total amount and the concentration of lipid and of calcium present in the inner coats of the arteries.

The index of morphological grading was obtained by determining the degree of stenosis produced by the largest plaque in the three major coronary arteries. This was done with use of a modification[4] of the criteria of Davis and Klainer.[5]

The index of plaque thickness was obtained by measuring the thickness of the intima at the point where it showed the greatest encroachment upon the lumen. This measurement was obtained by excising a small portion of the artery, embedding it in paraffin, staining sections with hematoxylin and eosin, and measuring the thickness of the intima with a micrometer eyepiece.

The indices of total lipid and lipid concentration were obtained by subjecting the remainder of the coronary arteries to alkaline storage and then estimating the total lipid by the method of Haven, Bloor, and Randall.[6] Prior to alkaline storage the

outer coats of the vessels, which contain fat, were stripped away and discarded.

The indices of total calcium and calcium concentration were obtained by subjecting an aliquot of the alkaline digest to acid digestion, after the method of Ma and Zuazaga,[7] and estimating the calcium by the Clark-Collip modification of the Kramer-Tisdall method as given by Hawk and associates.[8]

The estimations of the severity of disease, and the mast-cell counts, were carried out independently by different groups of workers. The members of one group determined the morphological grading and the thickness of the largest plaque, while those of a second group performed the tissue analyses. An additional worker, one of us (J. M.), carried out the myocardial mast-cell counts, and she did this without knowledge of the morphological or chemical findings in any of the subjects. The mast-cell counts were made exactly as described by Cairns and Constantinides.[2] Toluidine blue-stained 7μ paraffin sections were prepared from the formalin-fixed myocardium of each case. Two hundred high-power fields were counted from each sample, care being taken to avoid areas involved in fibrosis or infarction. The results were expressed as the average number of mast cells (± S. E. M.) per square centimeter of myocardium.

The final analysis of the data was made by comparing the myocardial mast-cell counts with the postmortem data after the latter had been segregated for persons into three natural groupings. The series was divided into three groups of crude morphological gradings (severe, moderate and slight) and into three groups of plaque thickness (greatest, intermediate and least), and a similar division was made for the other four indices. This division into groups was based on the natural range of levels for the number of cases available and was made without knowledge of the myocardial mast-cell counts. Finally, the intermediate (or moderate) group in each analysis was eliminated, and the mean myocardial mast-cell counts in the remaining two groups compared statistically with use of the "Student" t test.[9] P values of less than 0.05 were regarded as being significant, those equal to 0.05 were considered to be of border-line significance.

Observations

The comparison of the mean number of mast cells per square centimeter of myocardium with the severity of coronary atherosclerosis, as measured in six different ways, is given in Table 1. Although a trend toward decreasing cell counts with increasing severity of disease can be detected in some of the analyses, in none does any significant difference exist: the value for P in each comparison is greater than 0.05. It should be noted, however, that the comparison within the index of total lipid yields a P value that is very close to 0.05 ($t=1.92$).

TABLE 1.—*Myocardial Mast-Cell Counts with Different Grades of Coronary Atherosclerosis*

Index of Sclerosis	Mast Cells/Sq. Cm. of Myocardium, No.		
	Slight * Sclerosis	Moderate Sclerosis	Severe * Sclerosis
Morphological grade	348±43.0 † (12) ‡	320±28.8 (29)	291±30.1 (26)
Plaque thickness	328±34.8 (17)	313±26.9 (33)	310±41.6 (16)
Total lipid	325±30.4 (26)	355±30.3 (24)	237±32.1 (17)
Lipid concentration	356±33.0 (21)	300±27.6 (30)	285±38.9 (16)
Total calcium	323±30.0 (26)	333±30.8 (26)	265±37.0 (15)
Calcium concentration	330±27.9 (30)	300±28.5 (21)	325±29.1 (16)

* Severe cf. slight; all P values >0.05.
† Mean ± S. E. M.
‡ Number of cases.

Since aging has probably some influence on the severity of atherosclerosis, we have made a second comparison to eliminate this factor. Table 2 shows the myocardial mast-cell count and the severity of disease for each patient according to his age. Here, for the convenience of the reader, the mast-cell counts are arranged in decreasing order of magnitude for the patients in each age group. The data for each age decade are inadequate for statistical analysis, but by casual examination it is apparent that the myocardial mast-cell counts for the fatalities in any decade are not related to the severity of disease—the lowest counts are not found exclusively in patients with the severest grades of coronary sclerosis.

Finally, we have compared the mean myocardial mast-cell counts in patients who had pathological evidence of coronary occlusion (infarction, thrombosis, etc.) with the counts in patients who did not have these complications. Here a different, and apparently inconsistent, result was obtained. As shown in Table 3, the mast-cell counts in cases with complications are significantly lower than the counts in cases without com-

TABLE 2.—*Myocardial Mast-Cell Counts Versus the Severity of Coronary Atherosclerosis by Decades*

Autopsy No.	Age	Mast Cells/Cm.² Myocardium, No.	Morph. Grade	* Plaque Thickness	† Total Lipid	‡ Lipid Conc.	§ Total Calcium	‖ Calcium Conc.	¶ Complicating Lesions
A 81-55	33	175	+	+	+	++	+	+	No
A 18-54	40	541	+++	+++	++	+++	+	+	Yes (*b*)
A 5-55	43	454	++	+	+	+	+	+	No
A 71-55	46	428	++	++	+	++	+	+	No
A 86-54	54	620	+++	+	+	+	++	++	Yes (*c*)
A 7-54	54	489	+	+	+	++	+	+	No
A 40-54	59	375	+	+	++	++	+	+	No
A 116-54	59	148	++	+	+	++	+	+	No
A 55-53	55	122	+	+	++	++	+	+	No
A 81-54	57	105	++	+	++	++	++	++	Yes (*b*)
A 47-55	58	96	++	+	+	+	+	+	No
A 124-54	57	70	++	++	++	++	+	+	No
			+++	+++	+++	+++	+++	++	Yes (*b*)
A 83-54	62	663	++	++	++	++	++	++	No
A 17-54	64	602	++	++	++	+	++	+	No
A 60-55	62	532	++	++	++	+++	+	+	No
A 79-54	68	480	++	+++	++	++	+++	+++	No
A 103-54	64	454	+++	++	++	++	+++	+++	No
A 98-54	67	428	+++	+++	+++	+++	+++	+++	No
A 20-55	67	419	+++	+++	++	++	+++	+++	No
A 90-55	66	419	+	++	+	+	+	+	No
A 60-54	62	410	+++	++	+	+	++	+++	Yes (*b*)
A 14-54	60	393	+++	+++	+++	+++	++	+	Yes (*c*)
A 19-54	60	393	+	+	+	+	+	++	No
A 73-54	65	375	++	++	++	+	+	+	No
A 91-54	68	349	++	++	++	++	++	++	No
A 89-54	64	262	+++	+++	+	+	++	+++	Yes (*a*)
A 71-54	69	253	++	++	+	+	+++	+++	Yes (*b*)
A 75-53	64	228	++	++	+++	++	+++	+++	No
A 107-54	60	218	++	++	+	+	+	+	No
A 48-55	62	148	++	++	+	+	+	+	No
A 97-55	78	471	+	++	+	++	++	++	No
A 9-55	77	436	++	+	++	++	++	++	No
A 75-55	71	436	+++	+++	++	+	+++	+++	No
A 70-55	72	401	+++	+++	+++	++	++	++	No
A 17-55	74	393	++	+	+	++	+	+	No
A 96-55	76	393	++	+	+++	+++	++	+	No
A 58-54	76	349	++	+	+	+	+	+	No
A 102-55	76	332	+	+	++	++	+	+	No
A 3-55	79	323	++	++	+++	+++	++	++	No
A 91-53	70	314	+++	++	++	++	++	+	No
A 122-54	71	305	++	++	++	++	++	+++	No
A 15-54	73	297	++	++	+	+	+	+	No
A 68-53	75	291	+	+	+	+	+	+	No
A 123-54	71	279	+++	++	+++	+++	+++	+++	No
A 111-53	74	232	+	++	++	++	+	+	No
A 63-53	73	227	+	++	++	++	+	+	No
A 31-54	74	183	++	++	+++	+++	++	++	Yes (*c*)
A 88-55	77	166	+++	++	++	+++	++	++	No
A 40-55	72	148	+	+	++	++	+	+	No
A 38-54	70	131	++	+	++	+	+	+	Yes (*b*)
A 22-55	76	113	+++	+++	+++	+++	+++	++	Yes (*b*)
A 79-53	79	97	+++	++	+++	++	++	++	Yes (*b*)
A 52-55	76	87	+++	++	++	+	++	++	Yes (*b*)
						++	++	++	Yes (*b*)
A 72-54	83	646	+	++	+	+	+	+	No
A 76-54	85	471	++	+++	+	+	++	+++	No
A 84-55	87	410	+++	+++	+++	+++	+	+	No
A 93-54	82	349	++	++	+	+	++	++	No
A 25-55	85	340	++	+	++	+	++	++	No
A 2-55	88	323	+++	++	+	++	+	+	Yes (*b*)
A 14-55	85	236	++	++	++	++	++	++	No
A 112-54	84	227	++	++	+++	++	+++	+++	Yes (*a*)
A 114-54	83	200	+++	+++	+++	++	+++	++	Yes (*b*)
A 82-54	82	183	+++	+++	++	+++	+++	+++	Yes (*b*)
A 106-54	80	131	++	++	+++	+++	+++	+++	No
A 70-53	84	72	+++	+++	+++	+++	+++	+++	Yes (*a*)
A 82-53	81	59	++	+++	+++	++	+	+	Yes (*b*)
A 116-53	92	323	+++	++	++	+++	++	++	Yes (*b*)

* Plaque thickness in μ: + = <1000; ++ = 1000–2000; +++ = >2000.
† Total lipid in mg.: + = <50.0; ++ = 50.0–99.9; +++ = 100.0 and up.
‡ Lipid concentration in mg. %: + = <4.0; ++ = 4.0–5.9; +++ = 6.0 and up.
§ Total calcium in mg.: + = <10.0; ++ = 10.0–49.9; +++ = 50.0 and up.
‖ Calcium concentration in mg. %: + = <1.0; ++ = 1.0–2.9; +++ = 3.0 and up.
¶ Complicating lesions denotes presence or absence of pathological sequelae of coronary atherosclerosis: (*a*) a coronary thrombus or other occluding lesion; (*b*) a cardiac infarct; or (*c*) sudden death in association with severe coronary sclerosis but without an acute occlusion or infarct (acute coronary insufficiency).

TABLE 3.—*Myocardial Mast-Cell Counts in Cases With and Without Pathological Evidence of Coronary Artery Disease* *

	Cases, No.	Mast Cell Counts /Sq. Cm. of Myocardium	
With lesions	21	231±34.1	$P<0.001$
Without lesions	46	351±20.2	

* Pathological evidence of coronary artery disease was taken to be (1) a coronary thrombus or other acute occluding lesion; (2) a myocardial infarct, old or recent, and (3) sudden and unexpected death in cases with severe coronary sclerosis but without an occluding lesion or an infarct.

plications. The implications of this peculiar result are discussed below.

Comment

While our technique of measuring the severity of coronary sclerosis in autopsy material is more exact than that used by Constantinides, we realize that our individual indices are not without possible error. The difficulties inherent in morphological grading are well known to pathologists, and criticisms might also be leveled at our other indices of disease.[3] The situation being what it is, it would seem preferable to assess the condition of the coronary arteries in as many ways as possible and not by morphological grading only. In any event, we cannot accept Constantinides' group of cases that were without disease[2]; it is now generally recognized by investigators in the field of atherosclerosis that most adults have more or less of this condition.[10] Our failure to confirm Constantinides' observations that myocardial mast cells are less numerous in persons prone to severe coronary sclerosis can, therefore, probably be laid to differences in the technique of measuring the severity of disease. However, we have been able to demonstrate a highly significant reduction in the number of these cells in persons with cardiac infarction or other sequelae of coronary atherosclerosis. This seemingly inconsistent finding is due, of course, to the fact that occlusive phenomena may occur in association with moderate, or even slight, grades of coronary sclerosis; they are not confined to persons with severe grades of disease. It may also be pertinent to add that the complications of atherosclerosis are probably due to mechanisms that are quite distinct from those responsible for the primary atherosclerotic process (e. g., imbalance between supply and demand, capillary rupture with intimal hemorrhage, etc.). The question arises, however, does the local concentration of mast cells (and thus heparin) in or about an atherosclerotic plaque exert some inhibitory action on thrombus deposition within the adjacent arterial lumen? We doubt that this is so, since Pollak[11] has recently reported an absence of tissue mast cells in atherosclerotic plaques in general, and it is hard to envisage mast cells farther out in the arterial wall, or in the surrounding myocardium, exerting an effect on the endothelial lining of the artery.

The observations reported in this paper were made almost two years ago. Their publication, however, has been delayed because of our doubts, and the doubts of others, that mast-cell counts in human autopsy material were valid. No information was at hand to assure us that the number of mast cells did not change appreciably in the interval between death and the time when the tissues were immersed in fixatives —an interval which is quite variable in human autopsies. These doubts have now been resolved by the data that we have reported in the paper immediately preceding in this number of the ARCHIVES[12]: we have not found any significant change in the number of tissue mast cells in refrigerated material fixed within 24 hours after removal from the body.

Summary and Conclusions

The number of mast cells in the myocardium has been determined in 67 consecutive fatalities and compared with the severity of coronary atherosclerosis in the same cases. The severity of disease was measured independently with use of six different criteria. No relationship has been found to exist between the number of mast

cells and the degree of atherosclerosis. However, a significant reduction in the number of these cells was apparent in cases with complications such as thrombosis, infarction, and acute coronary insufficiency compared with the number in cases without these complications.

Mrs. Betty R. Cornish, Dr. E. C. Armstrong, Dr. J. B. Derrick and Mr. T. Moffatt assisted in measuring the severity of coronary sclerosis in the cases in this series.

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REFERENCES

1. Constantinides, P.: Mast Cells and Susceptibility to Experimental Atherosclerosis, Science 117:505, 1953.

2. Cairns, A., and Constantinides, P.: Mast Cells in Human Atherosclerosis, Science 120:31, 1954.

3. Paterson, J. C.; Cornish, B. R., and Armstrong, E. C.: The Serum Lipids in Human Atherosclerosis: An Interim Report, Circulation 13:224, 1956.

4. Paterson, J. C.; Cornish, B. R., and Armstrong, E. C.: The Gofman Indices in Coronary Sclerosis, Canad. M. A. J. 74:538, 1956.

5. Davis, D., and Klainer, M. J.: Studies in Hypertensive Heart Disease: Factors in the Production of Congestive Heart Failure, Am. Heart J. 20:98, 1940.

6. Haven, F. L.; Bloor, W. R., and Randall, C.: Lipids of the Carcass, Blood Plasma, and Adrenals of the Rat in Cancer, Cancer Res. 9:511, 1949.

7. Ma, T. S., and Zuazaga, G.: Micro-Kjeldahl Determination of Nitrogen: A New Indicator and an Improved Rapid Method, J. Indust. Engin. & Chem. (Analyt. Ed.) 14:280, 1942.

8. Hawk, P. B.; Oser, B. L., and Summerson, W. H.: Practical Physiological Chemistry, Ed. 12, Philadelphia, The Blakiston Company, 1947.

9. Fisher, R. A.: Statistical Methods for Research Workers, Edinburgh, Oliver & Boyd, Ltd., 1941.

10. Page, I. H.: Atherosclerosis: An Introduction, Circulation 10:1, 1954.

11. Pollak, O. J.: Mast Cells in the Circulatory System of Man, Circulation 16:1084, 1957.

12. Mills, J.; Strickland, G. and Paterson, J. C.: The Validity of Tissue Mast Cell Counts in Postmortem Material, to be published.

Pulmonary Megakaryocyte Studies in Rabbits

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These studies were undertaken in an effort to reproduce in rabbits an increase in the number of pulmonary megakaryocytes as has been observed in humans [1,2] and to determine what associated alterations may occur with adrenal stimulation in the number of blood platelets and possibly in the rapidity of blood coagulation. In an earlier investigation [1] we demonstrated evidence which appeared to indicate that the megakaryocytes usually formed in the bone marrow are transported to the lungs by the venous circulation, where on filtering through the pulmonary capillary bed they may break up into platelets. In the same study it could be demonstrated that in normal subjects, animal and human, usually 2-3 megakaryocytes could be seen in the lung capillaries of each square centimeter of pulmonary tissue. Thirty minutes after the subcutaneous administration of epinephrine in the rabbits, the lung sections were virtually emptied of the megakaryocytes and there occurred a simultaneous significant rise in platelets. It could also be demonstrated that larger numbers of pulmonary megakaryocytes were present in the majority of instances in humans dying of thromboembolic disease, of acute infections, in the near postoperative period, and from severe anemia.

It was postulated from the above that if large numbers of megakaryocytes are transported simultaneously to the lungs as a result of some bone marrow stimulation, under further stimulation the cells may disintegrate rapidly and there would result a sudden significant increase in circulating platelets.

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Since platelets are believed to liberate thromboplastin [2-4] as one phase of the blood-clotting phenomenon, it was considered desirable to determine whether the expected sudden increase in platelet formation could produce a hypercoagulability of the blood. Essential to this study, therefore, was the discovery of a means of increasing the number of pulmonary megakaryocytes in the experimental animal, the rabbit.

Materials and Methods

The majority of the animals used were 2-4 lb. young healthy female Belgian hares. In three instances adult hares of the same species weighing 7-10 lb. were used. The stimulating agents used were chiefly a 1:1000 solution of epinephrine, and since epinephrine is accepted as a stimulant of the adrenal cortex, an aqueous suspension of cortisone was also used in several instances. These solutions were administered subcutaneously. The smaller animals were given 10 minims (0.60 cc.) and the larger animals, 1 cc., of 1:1000 solution of epinephrine; 50 mg. of cortisone was administered to the smaller rabbits.

Platelet counts were performed by the indirect method of Fonio from freely flowing capillary-venous blood obtained from the rabbit ear. Direct counts were found to be impractical, owing to the small size of the rabbit platelets.

Coagulation times were performed in duplicate by the capillary tube method on freely flowing capillary-venous blood obtained from the rabbit ear. The Lee-White method was attempted, but its use was discontinued because of the difficulty in obtaining sufficient blood rapidly at the desired half-hour and hourly intervals.

Platelet counts and coagulation times were obtained before administration of the stimulating agent, 30 minutes after stimulation, and then at hourly intervals in most instances for a minimum of 4 hours. Where a second stimulation was given after the 4-hour interval, platelet counts and coagulation times were obtained 30 minutes later.

All animals were killed by rapid air embolism and autopsied, and lung blocks were immediately fixed in 10% formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

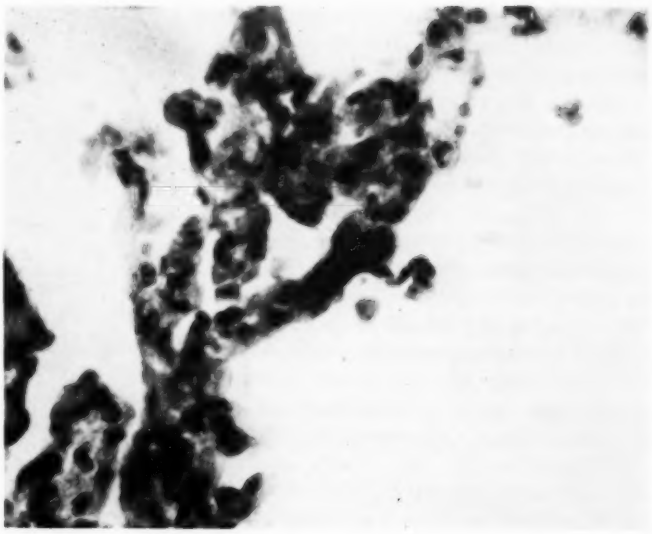

Fig. 1.—Megakaryocyte in a capillary vessel in the alveolar wall of a rabbit lung. Hematoxylin and eosin; reduced about 15% from mag. ×800.

Results

In the first study a total of 15 young rabbits was used. Eight were given 10 minims of the epinephrine solution, three were given 50 mg. of cortisone, and four used as controls were given a 1 cc. sterile saline solution. With two exceptions all animals were killed four hours after injection. The two exceptions were epinephrine-treated animals killed after six hours. With one exception the control animals revealed an average of 2-3 megakaryocytes per square centimeter of lung tissue section (Fig. 1). A slightly lower number was noted in the one exception. The epinephrine-treated animals all revealed a moderate to marked increase in pulmonary megakaryocytes, averaging 7-9 cells per square centimeter of lung tissue section, with the lowest being 3-5 cells and the highest, 12-14 cells per square centimeter. The latter was observed in one of two animals killed at six hours. The cortisone-treated animals averaged slightly lower than the epinephrine-treated animals, revealing 4-6 megakaryocytes per square centimeter of lung section.

Platelet counts were performed before and 30 minutes after epinephrine administration in 17 animals and then repeated at hourly intervals in 11 of the animals for 4 hours. Three additional animals treated with cortisone were followed with platelet counts for four hours. In two instances no significant elevation in platelets was detected at the 30-minute interval. All others, including the cortisone-treated animals, manifested an immediate significant in-

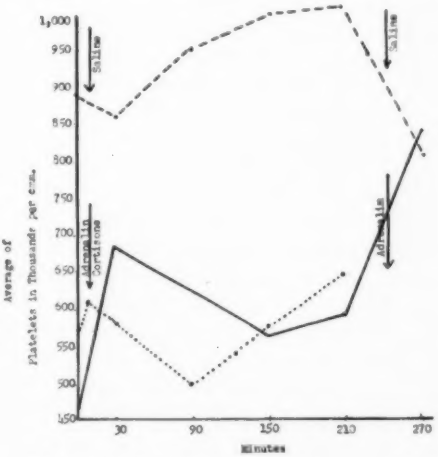

Fig. 2.—Graph of averaged platelet counts, demonstrating response to epinephrine and cortisone administration (solid line). Dotted line represents summertime controls. Interrupted line represents wintertime controls.

crease in platelets at the 30-minute interval, with a subsequent fall in platelets which continued to the 4-hour interval. The fall in platelets began in the majority of instances at the 90-minute interval. In most instances, the platelet counts returned to almost preinjection levels at the four-hour interval (Fig. 2). The cortisone-treated animals revealed a more sustained elevation in platelets during the four hours than did the epinephrine-treated animals.

In seven of the animals, a second subcutaneous administration of epinephrine was made after the 4-hour interval and platelet counts were performed again at the 30-minute interval. The counts all reached still higher levels than those reached after the initial epinephrine administration. Eleven animals were used as platelet controls, receiving 1 cc. of isotonic saline solution subcutaneously in place of the epinephrine solution. Four animals were given one administration of saline solution, with platelet counts obtained before and after injection and at regular hourly intervals for four hours. These revealed no significant alteration of the counts. Seven additional platelet control animals were given two injections of saline solution at the above-indicated intervals as for the test animals. Again no significant increase of platelets was noted after the saline administration. However, a marked difference in total platelets compared to the four control animals as well as the test animals was noted. Extremely high platelet counts were uniformly recorded in these seven control animals. This can only be explained by the one different condition for both control animal groups. All animals were raised and maintained under outdoor conditions and temperatures. The first control group, of 4 animals, as well as the test group, of 17 animals, were studied in midsummer. The second control group of seven animals were studied in midwinter, when very low temperatures were common.

Coagulation times were obtained on nine animals, performed simultaneously with the

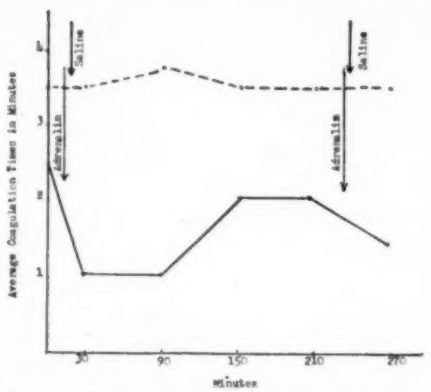

Fig. 3.—Graph of averaged coagulation times, demonstrating response to epinephrine administration (solid line). Interrupted line represents response of controls to isotonic saline solution.

platelet counts. In all but two animals a second injection of epinephrine was given at the four-hour interval and coagulation times were determined along with the final platelet counts. A significant shortening of the coagulation times was noted in all instances after the first administration of epinephrine. There followed a gradual prolongation of the coagulation time at the two-hour interval, which was sustained until the next injection of epinephrine, after the four-hour interval. After the second administration of epinephrine a less significant shortening of the coagulation time occurred (Fig. 3). The seven animals used above for platelet controls were also used for coagulation time controls, thus receiving two 1 cc. administrations of isotonic saline solution, with coagulation times performed with the platelet counts at the above-designated intervals. No significant alteration in coagulation times were obtained.

Comment

It appears that epinephrine stimulation in rabbits is capable of producing an increased pulmonary megakaryocytosis and a simultaneous increase in platelets and hypercoagulability of the blood. In a previous study [1] it was possible to demonstrate that the initial response to epinephrine stimula-

tion is a virtual emptying of the pulmonary capillaries of megakaryocytes, with a simultaneous increase in platelets. Within four to six hours there then occurs an increase in pulmonary megakaryocytes. The reason for the latter is not clear. However, a second administration of epinephrine after the four-hour interval appears to produce a still further increase in thrombocytes than results from the first epinephrine administration. This would appear to substantiate the earlier observation that four hours after the epinephrine stimulation there is present an increased number of pulmonary megakaryocytes. Therefore, a further increase in platelets is produced by the second epinephrine stimulation by breaking up larger numbers of megakaryocytes now present in the lungs. It would also appear possible that a marked thrombocytosis may be induced by subjecting the animals to low temperatures.

These studies appear to substantiate our earlier observations that pulmonary megakaryocytes may be a significant source of platelet formation. The studies also appear to indicate that an increased thrombocytosis may be associated with a hypercoagulability of the blood. The blood coagulation results obtained in this study are strikingly similar to those reported by Cannon and Gray,[6] who also used epinephrine as the stimulating agent in rabbits.

Summary and Conclusions

Epinephrine stimulation is capable of producing an increased pulmonary megakaryocytosis in rabbits. It may simultaneously produce an increased thrombocytosis and hypercoagulability of the blood.

Increased pulmonary megakaryocytosis on epinephrine stimulation may produce a further more marked thrombocytosis but a smaller degree of hypercoagulability of the blood.

These studies would appear to substantiate earlier observations that pulmonary megakaryocytes may be a significant source of platelet formation.

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REFERENCES

1. Sharnoff, J. G., and Kim, E. S.: Evaluation of Pulmonary Megakaryocytes, A. M. A. Arch. Path. 66:176-182, 1958.

2. Sharnoff, J. G.: Thrombotic Thrombocytopenic Purpura: A Report of 3 Cases, Am. J. Med. 23:740-747, 1957.

3. Biggs, R.; Douglas, A. S., and Macfarlane, R. G.: The Initial Stages of Blood Coagulation, J. Physiol. 122:538-553, 1953.

4. Ware, A. G.; Fahey, J. L., and Seeghers, W. H.: Platelet Extracts, Fibrin Formation and Interaction of Purified Prothrombin and Thromboplastin, Am. J. Physiol. 154:140-147, 1948.

5. Wright, I. S.: The Pathogenesis, Prevention and Medical Management of Peripheral Arterial Thrombosis, Am. J. Med. 23:704-712, 1957.

6. Cannon, W. B., and Gray, H.: Factors Affecting the Coagulation Time of Blood: II. The Hastening or Retarding of Coagulation by Adrenalin Injection, Am. J. Physiol. 34:225-231, 1914.

Production of Dystrophic Lesions in Skeletal Muscles of Dutch Rabbit by Diphtheria Toxin

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The possibility that lesions may arise in human skeletal muscles during acute infectious diseases has not been fully investigated. Reports of such lesions have appeared at various times since Zenker's histologic description of the skeletal muscles of cases dying from typhoid.[1] These reports indicate that a number of bacterial and viral infections are associated with lesions in voluntary muscles.[2] Of the infectious diseases, diphtheria has been most intensively investigated. However, the emphasis in such studies has been chiefly on the effects of diphtheria toxin on carbohydrate metabolism[3] and on neural function.[4,5] In only a few instances has there been an attempt to investigate the effect of this exotoxin on skeletal muscle.[6,7] These reports are in conflict as to the type and extent of such lesions, and there is no information available regarding functional alterations in skeletal muscle following injection into an experimental animal of diphtheria toxin. In the present study, an attempt was made to follow the effects of diphtheria toxin on the histology and physiology of mammalian skeletal muscle.

Materials and Methods

Immature Dutch rabbits, purchased from a local breeder, were used in these studies. All animals were maintained on a diet of Purina Rabbit Pellets and tap water before and after toxin injections. The toxin employed from the Lederle Laboratory, stock No. 42286-4733A, was a bacteria-free filtrate, and 0.0013 ml. was found by the probit method to equal one L. D_{50} for the experimental animals. In all experiments, the basic toxin dose (intraperitoneal) was 0.0056 ml. per kilogram of body weight. For the diaphragm studies, two separate groups of control animals were used: The first group was composed of normal rabbits not given injections, while the second group received injections of antitoxin neutralized toxin. The animals not given injections were killed for muscle studies at various times during the course of the experiment, while the toxin-antitoxin-treated rabbits were killed six days after injection. After a single toxin injection, experimental animals were studied at 8, 12, and 24 hours, and after toxin administration, at 6, 12, and 20 days. A portion of the diaphragm was removed, and maximum isometric tetanus tension and fatigue time were determined for this section of muscle. In general, the technique used was a modification of a method introduced by Bulbring.[8] The diaphragm slip was stimulated directly with use of a model S4A Grass stimulator output of 60 impulses per second, a delay of 0.04 msec., a pulse duration of 8 msec., and a voltage of 20. Results were recorded on an electrically driven kymograph, utilizing an isometric lever system. From these records, the maximum isometric tetanus tension and fatigue time, i.e., the time it took the tension to fall to 50% of the initial maximum value, were calculated. Wet and dry weights were obtained for each diaphragm slip, and tensions were calculated on the basis of the muscle wet weight. The adrenals were weighed, and a portion of the contralateral hemidiaphragm not used for testing, sections of the heart, and one adrenal were reserved for histological study.

The in vitro effect of diphtheria toxin was studied on isotonically contracting slips of diaphragm from normal rabbits. A slip from one hemidiaphragm was exposed to 0.022 ml. of toxin contained in 120 ml. of oxygenated Tyrode's solution for 60 minutes before and during the period of stimulation. A slip from the contralateral hemidiaphragm not exposed to diphtheria toxin was used as a control. Stimulation parameters in these

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experiments were as follows: frequency, 20 impulses per minute; delay, 0.04 msec.; impulse duration, 40 msec., and voltage, 25. The end-point of the experiment was a 50% decrease in the twitch height.

The in vivo effect of diphtheria toxin on the maximum isometric tetanus tension and fatigue time of the gastrocnemius was studied in a separate group of rabbits. Here, all animals were tested six days after the first injection of the basic toxin dose. Those rabbits receiving either two or three toxin injections were given injections at two-day intervals. On the sixth day after the first injection, the animals were anesthetized with pentobarbital sodium (30 mg. per kilogram) and ether. Dissection technique, myograph, and recording apparatus have been previously described.[9] The only modification was a photographic record of the maximum isometric tetanus tension and its subsequent decline with continued stimulation to 10% of its initial maximum value. Parameters of stimulation employed were a frequency of 100 impulses per second, a delay of 0.05 msec., a pulse duration of 0.7 msec., and a voltage of 30. After the functional studies were completed, the gastrocnemius was dissected out and weighed. Tensions were calculated on the basis of muscle wet weight. The adrenals were weighed, and sections of the contralateral gastrocnemius, diaphragm, heart, and one adrenal were preserved for histological study.

All tissues, except those adrenals and diaphragm sections which were to be examined for lipid content, were fixed in 10% formalin, dehydrated, embedded in paraffin, and sectioned at a thickness of 8μ. Hematoxylin and eosin was used as a routine stain. In order to reveal more fully differences among the muscles of the various experimental groups, Masson's trichrome was used for a connective tissue stain and phosphotungstic acid hematoxylin, for staining skeletal muscle cross striations. Frozen sections of unfixed adrenals and diaphragm samples from animals in the various experimental groups were stained with oil red O in order to compare the lipid contents with similar sections from control animals.

Statistical treatment of the data, where applicable, included comparisons among the various experimental and control categories, with use of the small sample theory t values.

Results

In Table 1 are listed the values obtained from the animals used in the study on the effect of injected diphtheria toxin on the contractility of diaphragm slips. No impairment of tension development by diaphragm slips from rabbits killed 8 and 12 hours after toxin injection was found. Though lacking statistical significance, the average tension value at 24 hours after injection was somewhat less (162 gm. of tension per gram of muscle) than the control value (191 gm. of tension per gram

TABLE 1.—*Average Values Obtained from Diaphragm Studies on Toxin-Treated Rabbits* *

Category	Animals, No.	Tension/Gm. Wet Weight	Fatigue Time, Sec.	Adrenal weights, Mg/100 Gm. Body Weight	Water Content, %
Control	29	191±9.2	19.1±1.0	10.3±1.0 (16 animals)	79.6±0.3
6 days after toxin-antitoxin injection	17	182±10.8 (0.55)†	17.8±1.2 (0.37)	10.9±1.5 (8 animals) (0.49)	79.1±0.3 (0.06)
8 hr. after toxin injection	20	192±19.0 (0.99)	22.0±1.5 (0.05)	10.9±1.1 (0.55)	78.9±0.6 (0.23)
12 hr. after toxin injection	10	201±21.4 (0.62)	21.6±0.9 (0.22)	12.5±1.6 (0.10)	80.0±0.3 (0.42)
24 hr. after toxin injection	10	162±4.6 (0.11)	20.7±1.5 (0.42)	11.8±0.5 (0.02)	78.6±0.5 (0.06)
6 days after toxin injection	21	106±12.7 (0.001)	17.0±1.0 (0.11)	17.6±1.0 (0.001)	81.1±0.4 (0.001)
12 days after toxin injection	9	88±33 (0.001)	13.7±3.7 (0.04)	11.1±1.0 (0.21)	81.8±0.5 (0.001)
20 days after toxin injection	9	109±20.3 (0.001)	16.9±2.5 (0.27)	11.2±0.8 (0.21)	82.0±0.5 (0.001)

* All columns include mean and S. E. M.
† Number in parentheses is P value. Statistical comparisons (P) in all instances are with the category labeled control.

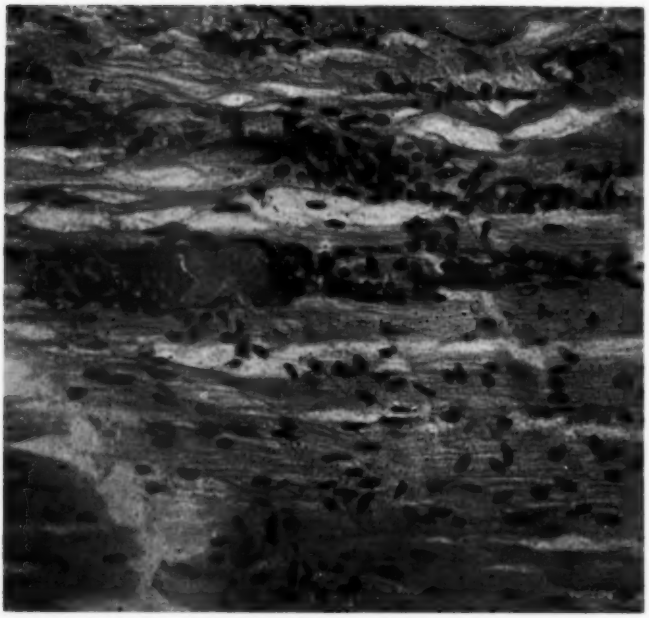

Fig. 1.—Diaphragm section taken from a rabbit 24 hours after injection of diphtheria toxin. Hematoxylin and eosin; reduced about 15% from mag. × 200.

of muscle). On the sixth day after toxin injection, the mean tension values showed a 45% decrease from control values (from 191 to 106 gm. of tension per gram of muscle ($P<0.001$). Functional recovery was not apparent at 12 or 20 days after injection, for the maximum isometric tetanus tensions recorded at these times were 88 and 109 gm. of tension per gram of muscle, respectively. These values were not statistically different from the tensions recorded in the six-day category but did differ significantly from the control value ($P<0.001$ for both categories). The fatigue times of the diaphragm slips in the various experimental categories were not significantly different statistically from control values.

Histological examination of the contralateral hemidiaphragms not used for tension studies generally supported the functional studies. No lesions were found in the diaphragms from animals in the 8- and 12-hour categories. Twenty-four hours after the toxin injection, the first structural lesions were seen (Fig. 1). Generally, the number of fibers involved in the degenerative reaction rarely exceeded 5% of the total number of fibers examined. Diaphragm specimens taken from animals killed six or more days after injection of the toxin displayed severe muscular lesions, i. e., a greater number of fibers were involved (Fig. 2).

Chronologically, the first muscle lesion observed was an abrupt loss of cross striations (Fig. 3). At this time, oil red O staining revealed numerous fine lipid droplets which gave a beaded appearance to the myofibril areas lacking striations. In none of these sections was fatty degeneration a prominent or conspicuous feature. Following loss of striation, the involved fibers became swollen and shortly thereafter, fragmented (Fig. 1). Accompanying the progressive degeneration and hyaline necrosis of the muscle fiber there was sarcolemmal nuclei proliferation and the appearance of numerous macrophages. In some sections this cellular reaction surrounded islands of necrotic tissue (Fig. 2); following removal of the necrotic material,

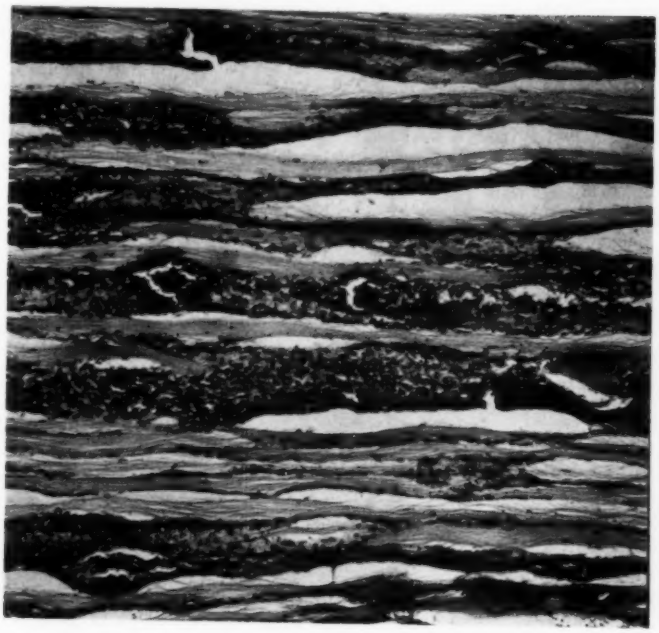

Fig. 2.—Diaphragm section taken from a rabbit six days after injection of diphtheria toxin. Hematoxylin and eosin; reduced about 15% from mag. × 80.

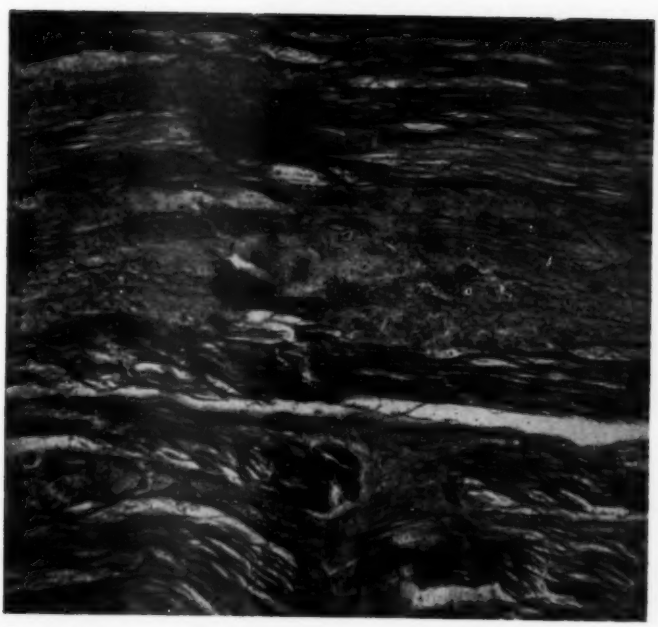

Fig. 3.—Diaphragm section taken from a rabbit six days after injection of diphtheria toxin. Phosphotungstic acid hematoxylin; reduced about 15% from mag. × 200.

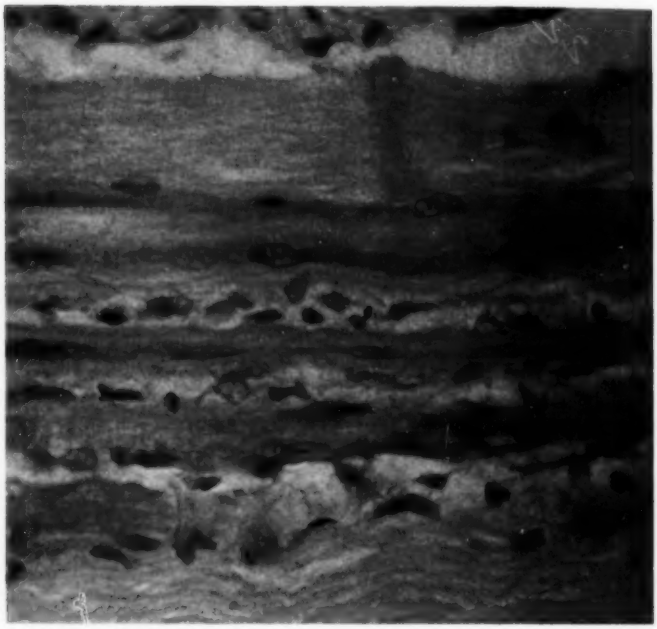

Fig. 4.—Diaphragm section taken from a rabbit 12 days after injection of diphtheria toxin. Hematoxylin and eosin; reduced about 15% from mag. × 400.

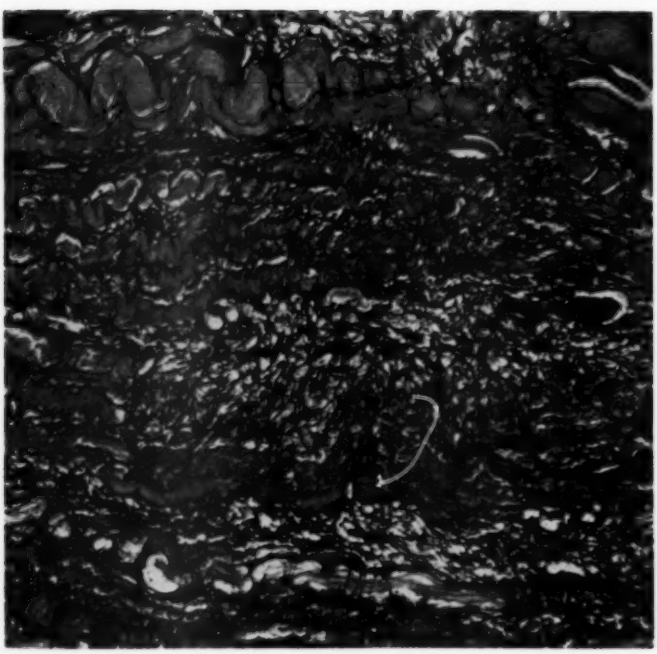

Fig. 5.—Diaphragm section taken from a rabbit 20 days after injection of diphtheria toxin. Masson's trichrome; reduced about 15% from mag. × 80.

TABLE 2.—*Effect of Toxin on Twitch Tension of Rabbit Diaphragm Slips* *

Category	Experiments, No.	Time, Min.
Control	12	119±22
Exposed to diphtheria toxin in vitro	12	102±21
P		0.56

* Average values together with standard errors for the time required during repetitive stimulation for the isotonic twitch tension of rabbit diaphragm slips to decrease to 50% of initial maximal values.

regeneration began (Fig. 4). This regeneration was not complete, since fibrous replacement of contractile tissue appeared early and became conspicuous during the later stages of attempted muscle repair (Fig. 5). Injection of neutralized diphtheria toxin did not cause any functional or histological alterations in the diaphragm. Cardiac muscle from all experimental animals was also examined microscopically. The lesions found were essentially as described for the diaphragm but involved much less contractile tissue.

The adrenal weights of the experimental animals did not differ greatly from control values, except at six days after toxin injection. At this time, hypertrophy was evident (Table 1). At 12 and 24 hours after toxin injection, the adrenals examined histologically for lipid content displayed in the zona glomerulosa an almost complete absence of material staining with oil red O. The zona fasciculata and zona reticularis appeared to contain a normal amount of substance with oil red O. With the dose of diphtheria toxin used, no evidence of a necrotic action on the adrenals was observed.

The diphtheria toxin in vitro had no apparent effect on the twitch height of normal diaphragm slips (Table 2).

In Table 3 are summarized the values obtained from studies made on the gastrocnemius muscles and adrenals of diphtheria-toxin-treated and control rabbits. The maximum isometric tetanus tension values after one injection (1481 gm. of tension per gram of muscle), two injections (1254 gm. of tension per gram of muscle), or three injections (1197 gm. of tension per gram of muscle) of toxin in a six-day period did not differ significantly from the control value (1297 gm. of tension per gram of muscle). Fatigue times (i. e., the time it took the tension to fall to 10% of the initial maximum value) for the gastrocnemii of animals given one injection (13.4 seconds) or two injections (12.7 seconds) of the toxin differed statistically ($0.03<P<0.05$) from the control value (15.9 seconds), while the average value for the gastrocnemii of animals given three injections (11.9 seconds) of the toxin significantly differed statistically ($P<0.001$) from the control value. Adrenal weights in all three categories of the gastrocnemius study were increased over the control value, and in all cases this increase was statistically significant ($P<0.001$).

Histologically, the lesions seen in the gastrocnemii never involved a large number of fibers. Usually the degeneration was restricted to less than 1% of the number of fibers in any one section. This contrasted sharply with the diaphragms of these same animals. Regardless of the number of toxin injections, all of the diaphragm sections had from 15% to 70% of their fibers involved in the degenerative reaction previously described.

TABLE 3.—*Summary of Mean Values and Standard Errors for Experiments on Gastrocnemius Muscles of Toxin-Treated and Control Rabbits* *

Category	Animals, No.	Tension/Gm. Wet Weight	Fatigue Time, Sec.	Adrenal Weights, Mg/100 Gm. b. w.
Control	18	1297±101	15.9±0.7	11.1±0.5
1 toxin injection in 6 days	9	1481±59 (0.37)	13.4±0.4 (0.03)	16.1±1.5 (0.001)
2 toxin injections in 6 days	9	1254±75 (0.78)	12.7±1.1 (0.03)	17.6±1.0 (0.001)
3 toxin injections in 6 days	19	1197±78 (0.49)	11.9±0.5 (0.001)	18.0±0.9 (0.001)

* Numbers in parentheses are *P* values.

Comment

Previous work on the effect of injected diphtheria toxin on skeletal muscle did not reveal the functional and histological changes noted in the present study. Instead of a fatty degeneration, as reported by other authors,[6,7] the lesions caused by diphtheria toxin in the present study more nearly resembled those found in experimental muscular dystrophy.[10] However, connective tissue replacement of contractile tissue indicates that the lesions in skeletal muscle caused by diphtheria toxin were not completely reversible. Warthin[11] noted fibrous replacement of contractile tissue in the heart after diphtheritic infection and suggested that "the possibility of cardiac fibrosis and impairment of cardiac function later in life must be borne in mind." The same precautionary statement may possibly be extended to skeletal muscle.

The early onset and character of the lesions noted in this study appears to rule out any immediate role of the nervous system in the production of such lesions. Waksman et al. found that evidence of diphtheritic polyneuritis did not appear until the second week after toxin injection.[5] The reason for the difference in the action of diphtheria toxin on these two types of tissue is not apparent but may be, as suggested by these authors, ". . . one of tempo, which must depend on the metabolic peculiarities of the particular cell." In the present in vivo studies, such a difference was evident when the action of diphtheria toxin on the diaphragm was compared with its action on the gastrocnemius.

The mechanism by which diphtheria toxin brings about structural lesions is not known. It has been suggested that the toxin interferes with the cytochrome system,[12] and evidence indicates that the toxin is capable of uncoupling oxidative phosphorylation.[13,14] It should be pointed out that the toxin need not necessarily penetrate into the cell to bring about such changes but may do so by altering, in some manner, the properties of the cell membrane. The difference in time of onset and degree of severity of the lesions would then not only be dependent upon the metabolic individuality of a cell but also upon any differences inherent in the cell membrane.

Summary

Muscle lesions were found in the Dutch rabbit after injection of sublethal doses of diphtheria toxin. A difference in the effect of the toxin on the functional and histological properties of the diaphragm and gastrocnemius muscles was observed. Functional as well as histological studies revealed that the muscle damage was prolonged in nature. The histological studies further showed that such lesions resembled those seen in experimental dystrophy, except for the large amount of fibrous replacement of muscle tissue during recovery from the effects of the toxin.

Department of Physiology, St. Louis University School of Medicine (4).

REFERENCES

1. Zenker, F. A.: Über die Veränderungen der willkührlichen Muskeln im Typhus abdominalis, Erlangen, A. E. Junge, 1863.

2. von Meyenberg, H., in Handbuch der speziellen pathologischen Anatomie und Histologie, edited by F. Henke and O. Lubarsch, Berlin, Springer-Verlag, 1929, Vol. 9, p. 259.

3. Holmes, E.: The Effect of Toxaemia on Metabolism, Physiol. Rev. 19:439, 1939.

4. Fisher, C. M., and Adams, R. D.: Diphtheritic Polyneuritis—a Pathological Study, J. Neuropath. & Exper. Neurol. 15:243, 1956.

5. Waksman, B. H.; Adams, R. D., and Mansmann, H. C., Jr.: Experimental Study of Diphtheritic Polyneuritis in the Rabbit and Guinea Pig: I. Immunologic and Histopathologic Observations, J. Exper. Med. 105:591, 1957.

6. Dudgeon, L. S.: A Study of the Various Changes Which Occur in the Tissues in Acute Diphtheritic Toxaemia, More Especially in Reference to Acute Cardiac Failure, Brain 29:227, 1906.

7. Jewesbury, R. C., and Topely, W. W. C.: On Certain Changes Occurring in the Voluntary Muscles in General Diseases, J. Path. & Bact. 17:432, 1913.

8. Bulbring, E.: Observations on the Isolated Phrenic Nerve Diaphragm Preparation of the Rat, Brit. J. Pharmacol. 1:38, 1946.

9. Schottelius, B. A.; Thomson, J. D., and Hines, H. M.: Capacity of Skeletal Muscle to Develop Isometric Tension After Prolonged Shortening, Am. J. Physiol. 179:491, 1954.

10. Pappenheimer, A. M.: On Certain Aspects of Vitamin E Deficiency, Springfield, Ill., Charles C Thomas, Publisher, 1948.

11. Warthin, A. S.: The Myocardiac Lesions of Diphtheria, J. Infect. Dis. 35:32, 1924.

12. Pappenheimer, A. J., Jr.: Bacterial Toxins, Fed. Proc. 6:479, 1947.

13. Pinchot, G. B., and Bloom, W. L.: Alterations in the Level of Muscle Phosphocreatine of Guinea Pigs Produced by the Injection of Diphtheria Toxin, J. Biol. Chem. 184:9, 1950.

14. Fonnesu, A., and Severi, C.: Phosphorylation Mechanisms in Cloudy Swelling, Experientia 10:28, 1954.

News and Comment

PERSONAL

Dr. William Boyd Gives Lecture.—Dr. William Boyd of Toronto gave the Arthur H. Sanford Lecture in Clinical Pathology at the annual meeting of the Minnesota State Medical Association, held in Minneapolis, May 22-24. He talked on the subject "The Spontaneous Regression of Cancer."

Dr. Paul R. Cannon Awarded Medal.—Dr. Paul R. Cannon, Chicago, was awarded the Groedel Medal at the annual meeting of the American College of Cardiologists, held in St. Louis, on May 22. He talked on the subject "The Changing Patterns of Disease."

Dr. Ernest W. Goodpasture Receives Award.—Dr. Ernest W. Goodpasture, scientific director of the Department of Pathology of the Armed Forces Institute of Pathology, was awarded the Gold-Headed Cane by the American Association of Pathologists and Bacteriologists at the 55th Annual Meeting of the Association, in Cleveland, in April.

Dr. Ernest W. Goodpasture Receives Medal.—Dr. Ernest W. Goodpasture received the Jessie Stevenson Kovalenko Medal at the spring meeting of the National Academy of Sciences, on April 28. The award was given for his outstanding contributions to medical science and to pathology.

Dr. Rene Jules Dubos Receives Award.—The Howard Taylor Ricketts Award for 1958 of the University of Chicago was presented to Dr. Rene Jules Dubos on May 12, 1958. Dr. Dubos lectured on the subject "Nutrition, Emotion, and Infection."

Dr. Esmond R. Long Gives Lecture.—Dr. Esmond R. Long, of the University of Pennsylvania, lectured before the International College of Surgeons on May 13, 1958, on the subject "Historical Facts About Morgagni, Rokitanski, and Virchow, Pathologists."

DEATHS

Dr. Henry Franklin Hunt.—Dr. Henry Franklin Hunt, of Danville, Pa., died on March 4, 1958. Dr. Hunt was a founding fellow of the College of American Pathologists and a former president of the American Society of Clinical Pathologists and the Pennsylvania Association of Clinical Pathologists.

ANNOUNCEMENTS

Translated Russian Journals.—Of the eight translated Russian journals published under contract with the National Institutes of Health of the U. S. Public Health Service, Oncology is being published for the calendar year 1957. It is published by the Pergamon Institute, 122 E. 55th St., New York 22, and is published with the financial support of the National Institutes of Health. It is offered by the Pergamon Institute at $30 a year.

Training Seminar in Diagnostic Use of Radioisotopes.—The second training seminar for pathologists in the diagnostic use of radioisotopes, sponsored by the American Society of Clinical Pathology and the Oak Ridge Institute of Nuclear Studies, is to be held at the Medical Division, Oak Ridge, Tenn.

A training seminar on the diagnostic use of radioisotopes has been set up specifically for certified pathologists and designed to meet the recommendations and requirements of the Atomic Energy Commission. This seminar will furnish a reasonable introduction to radioisotopes for those pathologists who are principally interested in diagnosis but who may be called on to assist and advise on therapeutic uses of radioisotopes. The program will consist of two one-week meetings separated by a three-month interval. The first (basic) week will consist mainly of fundamental techniques and concepts. The second (clinical) week will be held approximately three months after the first and will be devoted mainly to the practical

application of these principles. It is expected that in the intervening three months the participating pathologists will visit laboratories in their home areas which are already approved for the use of isotopes and will assimilate relevant literature. At the end of the second week the Medical Division of ORINS will give to participants a statement of preceptorship in the diagnostic uses of radioisotopes. It is emphasized that while this does not automatically qualify any person for AEC permission to use isotopes, the course is designed to meet the training requirements of the Isotope Extension of the AEC.

The training seminar will consist of approximately 50% lectures and demonstrations and 50% laboratory work. Each participant will be expected to administer to himself a dose of less than 10μc. of I^{131} to accomplish a thyroid-uptake or a blood-volume measurement.

The teaching staff has been selected from pathologists, internists, surgeons, radiologists, physicists, and biochemists in and around Oak Ridge or members of the Council on Radioisotopes, ASCP. All are experienced in the clinical use of radioisotopes.

The two one-week programs are considered as a unit, and application must be made for both. The first week will be held Sept. 22-27, 1958; the second week, Dec. 8-13, 1958.

Requirements.—Applicants must be certified in either clinical pathology or pathologic anatomy or be members or fellows of the American Society of Clinical Pathology; they must be United States citizens and must have a license to practice medicine.

Fee.—Entire course, $25, payable to the Oak Ridge Institute of Nuclear Studies on the first Monday.

Application.—Application should be made to Dr. Oscar B. Hunter Jr., Chairman, Council on Radioisotopes, American Society of Clinical Pathology, Suite 1000, Columbia Medical Building Annex, 915 19th St., N. W., Washington 6, D. C. Applications will be reviewed by the Council on Radioisotopes, ASCP. Twenty qualified candidates will be accepted in chronologic order. Information regarding housing will be supplied with the notices of acceptance.

Books

Clinical Laboratory Methods. Fifth edition. By W. E. Bray. Price, $9.75. Pp. 731, with 124 illustrations. C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1957.

The fifth edition of this very useful book has been printed in a smaller format for the sake of convenience and with no loss of content. Among the outstanding features is the section entitled "significance," which is included with each test, giving the interpretation of the laboratory findings. One chapter is given over to an alphabetical listing of all reagents used. The present edition includes new charts and figures as well as many new tests. For example, the use of radioactive iodine and protein-bound iodine in the diagnosis of thyroid disorders, the mercurimetric method for blood and urine chlorides, and spectrophotometric methods for the determination of blood sugar, cholesterol, urea, urine phosphorus, and blood phosphatase are included, to mention only a few.

The book has proved useful in many laboratories, and the new edition would be a valuable adjunct to any clinical laboratory.

Science Looks at Smoking. By Eric Northrup. Introduction by Dr. Harry S. N. Greene. Price, $3. Pp. 190. Coward-McCann, Inc., 210 Madison Ave., New York 16, 1957.

The title is unfortunately a misnomer, for the book has very little to do with science if by that term we mean an impartial search for truth. Instead, this little volume represents special pleading. It considers a small part of the evidence that smoking may be harmful. By attacking this evidence the author incidentally points out some of the fallacies of statistics and the obscurity surrounding the word "cause." The book can be recommended to those who are already firmly resolved not to give up smoking.

Methods in Surgical Pathology. By Henry A. Teloh, M.D. Price, $4.75. Pp. 127, with 12 illustrations. Charles C Thomas, Publisher, 301-327 E. Lawrence Ave., Springfield, Ill., 1957.

This monograph outlines in logical step-wise fashion most of the procedures followed in the surgical pathology laboratory. The first chapters consider the gross examination and description of surgical specimens, the technique of taking blocks, and the methods of frozen sections. The importance of adequate records and index files is discussed. Several pages are devoted to the problems of prognosis, and here the function of the surgical pathologist in determining future potentialities of disease processes is emphasized. The remainder of the book considers, in turn, the various anatomical specimens as they are submitted to the surgical pathology laboratory. Each section starts with a general discussion of the specimen and the special problems that may be encountered in the material. Then follows the procedure for examination of the particular specimen. These sections cover all of the routine surgical pathology material, with the exception of neurosurgical specimens.

The particular value of this book is in the surgical pathology laboratory that is training residents. The discussions are simple, straightforward, concise, and well written. This becomes particularly valuable when an inexperienced resident first enters a busy surgical pathology laboratory. In just a few minutes the resident can learn the problems presented by a particular specimen and should be able to perform an adequate examination and description of it. Later he will need less reference to this book as he learns the problems of the surgeon and the questions which must be answered on each specimen. The experienced surgical pathologist will find nothing that is new for himself, but he will find this book an excellent aid in his resident training program.

Fifth Meeting of l'Association des Sociétiés européennes et méditerranéenes de gastro-entérologie, London July 18-21, 1956. International Congress of Gastroenterology. Edited by Harold Edward. Price, $18.70. Pp. 782, with numerous illustrations. S. Karger AG., Arnold Böcklinstrasse 25, Basel, 1956.

This is a large and beautifully edited volume of reports and contributions of research men in the field of gastroenterology from many different countries. The first section comprises papers on the physiology and pathology of the lower end of the esophagus; knowledge of this region has greatly increased in recent years, and the array of papers on this subject covers

most of the information accumulated lately. The study by the Mayo group, with the use of a miniature electromagnetic transducer, is a real contribution to the understanding of the physiologic sphincteric mechanism existing at the lower end of the esophagus. The cineradiography films presented by Porcher promise new information on the continuous sequence of events in the esophagus during the act of deglutition. A chapter follows with papers on premalignant conditions of the alimentary tract, a subject of high interest in view of the possible applicability of newer methods of early detection. The paper on cytology of the colon in ulcerative colitis stresses the difficulties in diagnosing cancer when an inflammatory process is present. The second part of the short articles contains important papers in the field of peptic ulcer and cirrhosis of the liver. Part 3 contains contributions in chronic ulcerative colitis. Thirty-six papers contributed by researchers from sixteen different countries deal with numerous aspects of this difficult and important problem. Some of the contributions are of fundamental value and constitute a real addition to our knowledge of the pathology of ulcerative colitis. The papers by Bockus and associates and by Cullinan and MacDougall are particularly noteworthy. Others are products of personal limited experience and may underline the singular differences in severity and importance of the disease in different geographical areas. The foreword by Dr. Thomas Hunt and the closing remarks by Professor Gasbarrini beautifully stress the importance of this international gathering for the progress of knowledge and for the development of a "brotherhood, based on eternal spiritual principles." This volume will be a valuable acquisition for all those interested in gastroenterology.

Physical Techniques in Biological Research: Vol. II. Physical Chemical Techniques. By Gerald Oster and Authur W. Pollister. Price, $12.80. Pp. 502, with 143 illustrations. Academic Press Inc., 111 5th Ave., New York 3, 1956.

This well-conceived volume, the second in a series, presents various physical chemical techniques used in biology and will serve to fill the artificial gap that has occurred between physical chemistry and biology in the minds of many. The nine chapters cover the following subjects: tracer techniques; the measurement and properties of ionizing radiation; sedimentation, diffusion, and viscosity; surface film techniques; adsorption and chromatography; electrophoresis and ionophoresis; electrical potential differences; magnetic methods, and x-ray diffraction and scattering. Each contributing author introduces the theoretical basis of the method and then describes apparatus, manipulations, and applications of biological interest. Consequently, a composite picture of the technique is made available so that its applicability and usefulness to one's field of interest can be ascertained. The chapters are well written and have exhaustive references and excellent illustrative material.

An Atlas of Muscle Pathology in Neuromuscular Diseases. By J. Godwin Greenfield, M.D.; G. Milton Shy, M.D.; Ellsworth C. Alvord Jr., M.D., and Leonard Berg, M.D. Price, $9. Pp. 104, with 90 illustrations. The Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1957.

It is both a pleasure and a satisfaction to have this small but extremely informative monograph as one of the last momentoes of the thoughtful industry of the senior author, whose recent death has been the occasion for sorrow all over the world.

The book is divided into two separate parts, the first dealing only with the fundamental and unequivocal pathological changes encountered in muscle, apart from any consideration of etiology or pathogenesis and the second, with clinicopathological correlations. In the first section, interpretive prejudicial terms, such as *necrosis* and *inflammation*, are avoided. Instead, the changes are classified by simple purely descriptive labels, such as *cloudy and granular change:, basophilic fibers with vesicular nuclei*, and *leucocytic infiltrations*. Similarly, the abnormal clinical states of muscle with which the pathological alterations are later correlated are classified in four broad groups (rather than by conventional but biased names like *dystrophy* or *myositis*), as follows: (1) *distal muscular syndromes (exclusive of myotonic syndromes) and other possibly neurogenic disorders;* (2) *myotonic syndromes;* (3) *proximal muscular syndromes*, and (4) *myasthenia gravis*. Pathologists and clinicians alike may object that the classifications are vague and without significant meaning. Nevertheless, as used by the authors, they make possible a freshness of viewpoint and a start-from-scratch approach greatly needed in this generally neglected and confused area of pathology.

The book is primarily an atlas, and there can be no disagreement about its usefulness as such. The clear brief pathological descriptions are illustrated by superb photomicrographs, more than half of which are excellent color reproductions.

Nomina anatomica. Fifth edition. By Dr. Fr. Kopsch. Revised by Dr. K-H. Knese. Price, $1.55. Pp. 155. Georg Thieme Verlag, Herdweg 63, (14a) Stuttgart N, 1957.

The Sixth International Congress of Anatomists, meeting in Paris, in 1955, adopted a new revision of anatomical nomenclature. This action has prompted the authors to revise previously issued alphabetical lists of anatomical terminology based on the Basle "Nomina anatomica" of 1895 (B. N. A.) and the Jena "Nomina anatomica" of 1935 (J. N. A.) to include the recently adopted nomenclature. This volume should be of particular value to authors, editors, teachers, and students during the foreseeably long transition period when all three terminologies will be used in the medical literature.

Pediatric Cardiology. By A. S. Nadas. Price, $12. Pp. 587, with 343 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1957.

As knowledge of clinical and pathological aspects of the cardiovascular system increases separate areas become so complex that they require full-time study and investigation. Heart disease in children has developed into such an area, and the twenty-year period between the publication of Maude Abbott's "Atlas of Congenital Heart Disease" and Nadas' "Pediatric Cardiology" demonstrates this.

Nadas calls his book a handbook for the pediatrician, but it is rather a review of the current status of cardiology in children. It represents a clinical pathological approach to the subject and is a book for the clinician; but pertinent and adequate physiological details are included.

The book is divided into four parts. Part 1 is a description of diagnostic adjuncts, including phonocardiography, angiocardiography, and catheterization. Part 2 considers acquired heart disease, especially rheumatic heart disease and the cardiac arrhythmias. Part 3 presents superbly the clinical and pathological physiology of congenital heart disease and represents a summary of Nadas' extensive experience at the Children's Medical Center, in Boston. Concise but somehow adequate consideration is given to all of the major clinical problems presented by the varied and complex pathology of congenital cardiac defects. Part 4 considers anesthesia for children with heart disease, in relation to both cardiac surgery and noncardiac surgery.

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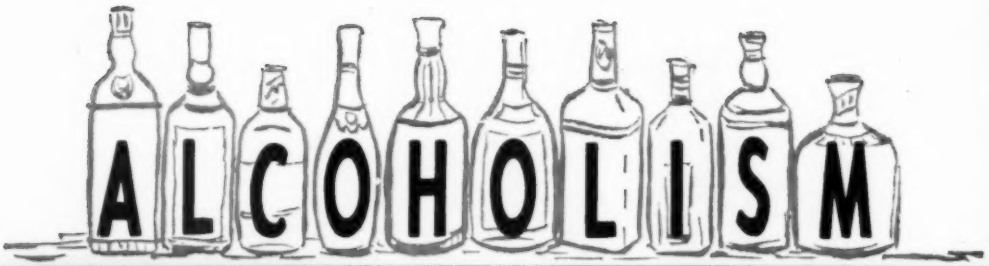

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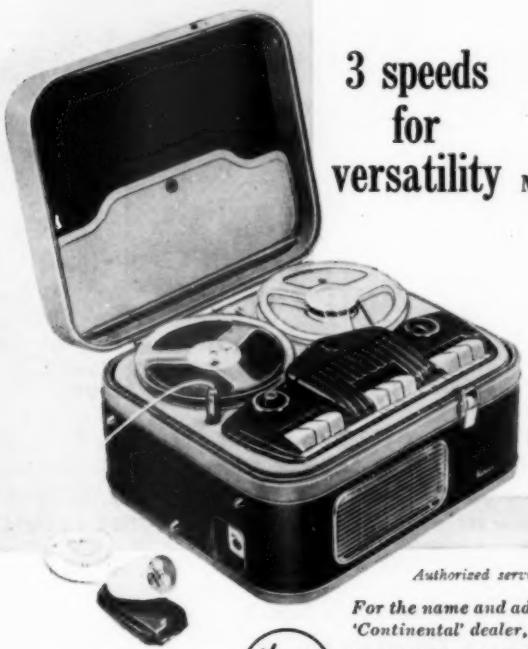

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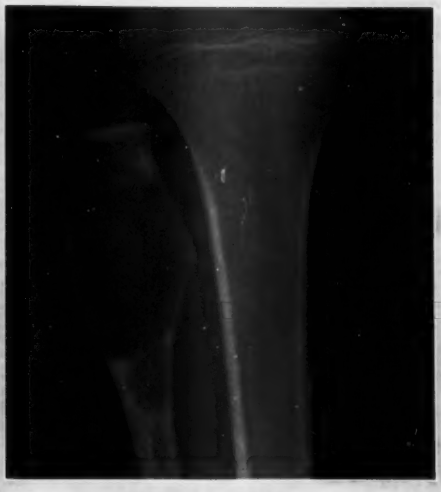

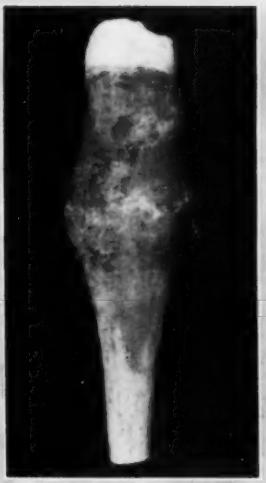

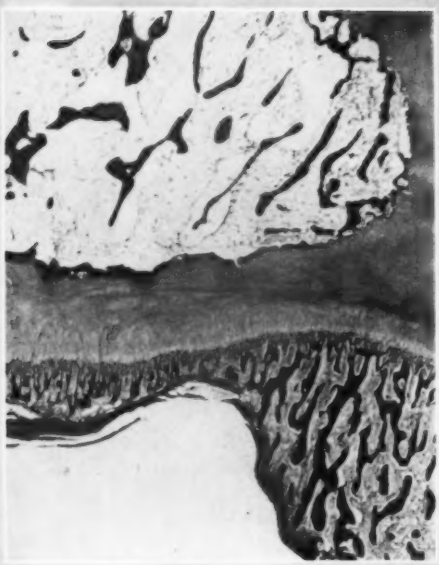

BONE CYST . . . 4 aspects

Radiograph *(upper left)* of bone cyst of the fibula. The lesion has its characteristic location in the end of the shaft and does not transgress the plate.

Photograph *(upper center)* of external surface of upper end of fibula resected because of the presence of a bone cyst. The contour of the cystic area is expanded, and two healed fracture lines are to be noted.

Photograph *(upper right)* of transected specimen showing the lesion illustrated above, center. The cyst cavity contains considerable organizing blood, which was present because of recent fracture.

Photomicrograph (x 3) *(left)* showing topography of the fibular cyst. From above down, one notes epiphysis, epiphyseal cartilage plate, and cyst wall abutting upon the metaphyseal spongiosa and plate area.

For data on Nonossifying Fibroma, turn page.

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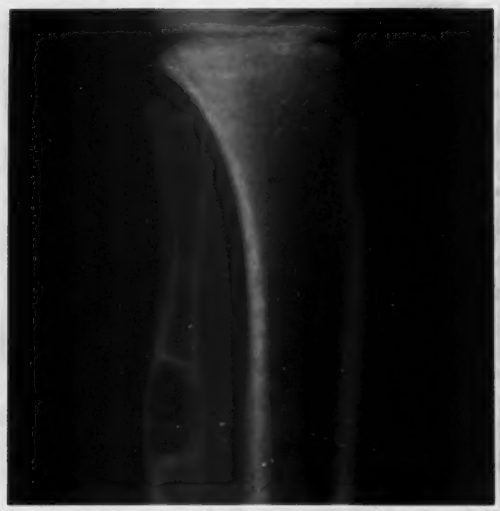
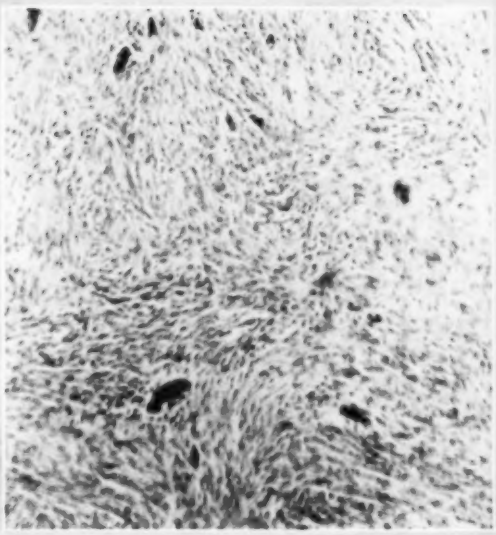


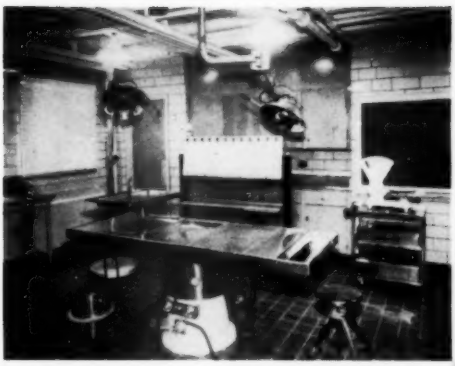
NONOSSIFYING FIBROMA . . . 3 aspects

Radiograph *(upper left)* of a nonossifying fibroma in the shaft of a fibula of a boy of 8 years. The lesional area appears somewhat loculated.

Photograph *(upper right)* of segment of fibula (cut longitudinally) containing the lesion shown in the preceding radiograph.

Photomicrograph (x 15) *(right)* showing the general pattern characteristic of the lesional tissue of nonossifying fibroma. The stromal cells are spindle-shaped and whorled, and intermingled with small numbers of giant cells.





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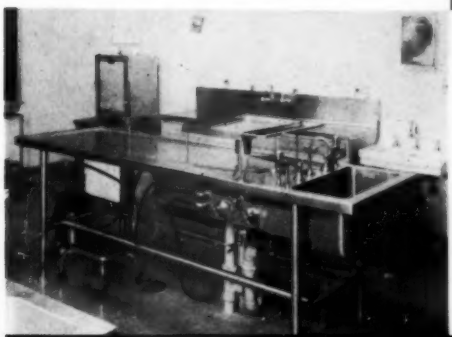

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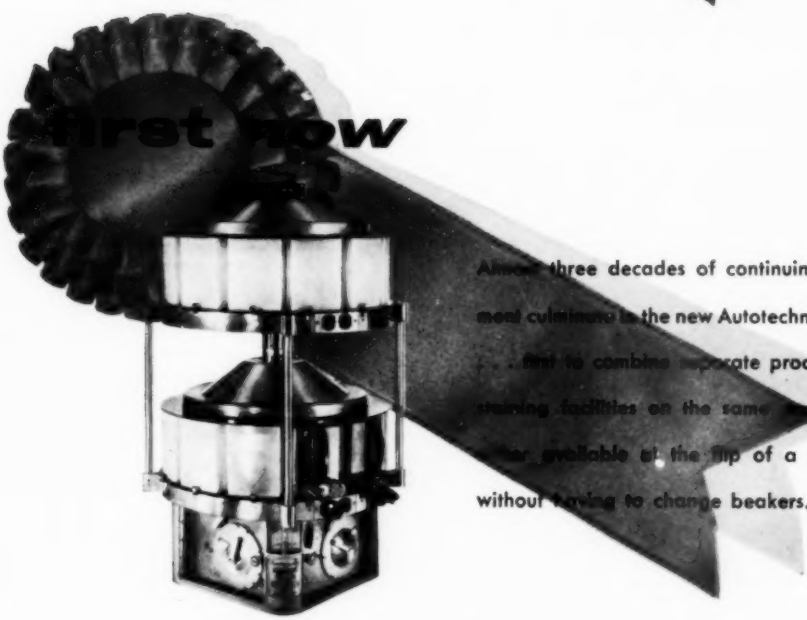

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